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CANADIAN JOURNAL OF RESEARCH

VOLUME ~~18~~ *19*
1940

SECTION D



CANADA

Published by the
**NATIONAL
RESEARCH COUNCIL
of CANADA**

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

JANUARY, 1940

NUMBER 1

STUDIES ON CERCARIAL DERMATITIS AND THE TREMATODE FAMILY SCHISTOSOMATIDAE IN MANITOBA¹

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Abstract

A biological investigation of cercarial dermatitis in Manitoba has revealed that three species of apharyngeal brevifurcate cercariae are responsible for the disease. An ecological study of the organisms and their molluscan hosts has been carried out, and descriptions of the general types of habitats are given in addition to the distribution of the organisms. A synopsis of the dermatitis-provoking cercariae and the adult schistosomes of the world is given, and the classification of the adults is revised in part. The results of a survey of mammals and birds for adult schistosomes and also the results of experimental exposures of animals are included. A new species of apharyngeal brevifurcate cercaria and two new species of *Ornithobilharzia* are described. The description of *Cercaria wardlei* McLeod, 1934, is revised.

Introduction

An investigation of the etiology of swimmer's itch was undertaken in Manitoba in 1933, and the work has been carried on periodically since that time. Prior to the beginning of the work reported for the first time herein, it had been found that this local transitory skin disease of man was identical with the so-called cercarial or schistosome dermatitis in parts of the United States and various other countries. It had also been found that the disease in this province is provoked by the penetration of the skin by one or more species of brevifurcate larval trematodes or cercariae; that the organisms and their snail hosts are abundant in Clear Lake; that three species of adult schistosomes occur locally in wild ducks and a fourth species occurred in Herring Gulls collected near Yarmouth, Nova Scotia; also that a number of cercariae had failed to penetrate into, or reach maturity in, several species of experimental mammals and birds.

To elucidate the biology of the organisms concerned and bring our understanding of them to a point where control measures of some value might be suggested, the work has been continued and expanded. The purpose of the problem reported on herein, therefore, has been:—

1. A survey of southern Manitoba for dermatitis-provoking cercariae and their molluscan hosts.

¹ Manuscript received in original form March 29, 1939, and as revised September 8, 1939.

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2. A brief study of the ecological factors responsible for their abundance and distribution.
3. A continuation of the survey of birds and mammals in an effort to discover what adult schistosomes occur in this area.
4. Exposure of experimental mammals and birds to the cercariae in an attempt to complete the life-history stages.
5. A study of related but non-dermatitis-provoking cercariae.
6. A study of the literature on larval and adult schistosomes of the world to determine the relationships of the local forms.
7. A revision of the classification of the Schistosomatidae.

Historical

The phylogeny of the schistosomes is not clearly understood at the present time, but the parasitic habit of life among them is apparently one of long standing. That the adults of one species have been the inhabitants of the blood vessels of man for at least 30 centuries has been proved beyond doubt by Ruffer (129). The first species of schistosome was discovered by Rudolphi in 1819 and was of the so-called non-human type, being a parasite of birds. *Schistosoma haematobium*, the second species recorded, was a true parasite of man and was discovered by Bilharz in Egypt in 1852. It was first recorded as *Distoma haematobium* but received its present name from Weinland in 1858. The second schistosome of man, *S. japonicum*, was described by Katsurada in 1904, and a third, *S. mansoni*, also well known at the present time, by Sambon in 1907.

In the interval between 1819 and 1912 thirteen species of schistosomes were recorded, but at that time nothing was known regarding the life-histories of the organisms or their method of gaining entrance to the host. It may be noted, however, that in 1896 Looss suggested it might be by active penetration of the skin.

The species recorded during this interval and the type of host in which each was found are as follows:—

Ornithobilharzia canaliculata (Rudolphi, 1819) Odhner, 1912, from birds.

Schistosoma haematobium (Bilharz, 1852) Weinland, 1858, from man.

Schistosoma bovis (Sonsino, 1876) Blanchard, 1895, from mammals and man (?).

Bilharziella polonica (Kowalewski, 1895) Looss, 1899, from birds.

Ornithobilharzia kowalewskii (Parona and Ariola, 1896) Odhner, 1912, from birds.

Dendritobilharzia pulverulenta (Braun, 1901) Skrjabin, 1924, from birds.

Schistosoma japonicum Katsurada, 1904 (*S. cattoi* Blanchard, 1905), from man.

Schistosoma indicum Montgomery, 1906, from mammals.

Schistosoma spindalis Montgomery, 1906, from mammals, rarely man.

Ornithobilharzia bomfordi (Montgomery, 1906) Price, 1929, from mammals.

Schistosoma mansoni Sambon, 1907, from man.

Gigantobilharzia acotylea Odhner, 1910, from birds.

Ornithobilharzia intermedia Odhner, 1910, from birds.

In the years 1913–14, Miyairi and Suzuki demonstrated that in the case of *S. japonicum* a parthenogenetic stage is passed through in a molluscan host and that the cercarial larvae so produced gain entrance to the appropriate warm-blooded host by active penetration of the body surface. This cleared the way for further work and the chronology of events from then up to the present time is as follows:—

Schistosoma japonicum, life-history by Miyairi and Suzuki, 1913.

Ornithobilharzia turkestanicum (Skrjabin, 1913) Price, 1929, from birds.

Schistosoma haematobium and *S. mansoni*, life-histories by Leiper, 1915.

Austrobilharzia terrigalensis Johnston, 1917, from birds.

Schistosoma spindalis, life-history by Soparkar, 1918.

Trichobilharzia ocellata (Skrjabin and Zakharow, 1920) Brumpt, 1931, from birds.

Schistosomatium pathlocopticum Tanabe, 1923, from mammals, life-history by Tanabe, 1923.

Macrobilharzia macrobilharzia Travassos, 1923 (Price, 1929, 1931), from birds.

Ornithobilharzia odhneri Faust, 1924, from birds.

Dendritobilharzia loossi Skrjabin, 1924, from birds.

Schistosoma incognitum Chandler, 1926, from man.

Schistosoma bovis, life-history (?) by Faust, 1926.

Pseudobilharziella yokogawai (Oiso, 1927) Ejsmont, 1931, from birds. Life-history by Oiso, 1927.

Ornithobilharzia, 2 sp. Linton, 1928, from birds.

Schistosoma faradjei Walkiers, 1928, from man (?).

Schistosoma mattheii Le Roux, 1929, from mammals.

Macrobilharzia ankingae (Price, 1929) Price, 1931, from birds.

Heterobilharzia americana Price, 1929, from birds.

Microbilharzia chapini Price, 1929, from birds.

Pseudobilharziella kowalewskii Ejsmont, 1929, from birds.

Schistosomatium douthitti H. F. Price, 1929, from mammals.

Ornithobilharzia pricei Wetzel, 1930, from birds.

Schistosomatium douthitti, life-history by Price, 1931.

Schistosoma rodhaini Brumpt, 1931, from mammals.

Schistosoma cucassoni Brumpt, 1931, from mammals.

Trichobilharzia ocellata, life-history by Brumpt, 1931.

Bilharziella polonica, life-history by Brumpt, 1931.

Bilharziella margebowiei Le Roux, 1933, from mammals.

Schistosoma nasalis Rao, 1933, from mammals.

Schistosoma suis Rao, 1933, from mammals.

Schistosoma intercalatum Fischer, 1934, from mammals and man.

Microbilharzia manitobensis McLeod, 1936, from birds.

Microbilharzia canadensis McLeod, 1936, from birds.

Microbilharzia lari (Syn. *Ornithobilharzia lari*) McLeod, 1937, from birds.

Pseudobilharziella querquedulae McLeod, 1937, from birds.

Ornithobilharzia sp. Young, 1937, from birds.

Exclusive of the two species reported for the first time in this thesis, this makes a total of 25 species known to infest birds. Of these only three life-histories have been completed. There are also 18 species from man or other mammals, of which seven life-histories are known.

Fourteen of the above 43 species were first recorded from North America and an additional one, *Bilharziella polonica*, originally described from Poland, has since been found on this continent by Price (114). Creech and Miller (45) encountered cases of nasal granuloma in cattle in Louisiana, U.S.A., with symptoms identical to those occurring in India; but the causal organism, later described by Rao (124) as *S. nasalis*, was not recovered in America. The life-histories of *Schistosomium pathlocopticum* and *Schistosomium douthitti*, both originally described from America, are known. They spend their adult stages in rats and mice, Tanabe (149), H. F. Price (116). *S. douthitti* has also been found in wild muskrats (*Ondatra zibethica*) in Michigan and Minnesota by Penner (117).

The three common species of human schistosomes, undoubtedly, have been introduced many times into North America, but that they never have become established seems to be fairly certain. This may be due to existing sanitary conditions, absence of suitable molluscan hosts, or unfavourable climatic conditions.

Cercarial Dermatitis

In many countries including Canada and the United States, where native schistosomiasis is unknown or endemic, the occurrence of a transitory skin disease, popularly known as "swimmer's itch" or "cercarial dermatitis", has been known for many years. A true causal organism was first associated with it by Cort (36, 37) in Michigan, and the condition is now widely recognized to be the result of attacks by schistosome larvae. For some time the disease was thought to be confined to man, but a case in dogs has been reported recently by Herber (59). In addition, the writer has information to the effect that in earlier times on the western prairies, cattle wading in sloughs were frequently afflicted with a dermatitis on the legs and belly.*

Following the report of Cort's discovery, numerous authentic cases of cercarial dermatitis, that previously would have been attributed, no doubt, to attacks of chigoes, chiggers, larval hookworms, various insects, or put down to unknown causes, were reported. Christensen and Greene (26) reported cases from lakes in the vicinity of Minneapolis, Minnesota. Here the causal organism was found to be a schistosome larva whose adult stage is, even yet, unknown and which had been tentatively named *Cercaria elvae* by Miller (96, 97).

*Personal conversation with Mr. Stuart Criddle, Treesbank, Man.

Matheson (88) reported an outbreak among bathers at an artificial lake at Cardiff, Wales. He considered a species of furcocercous cercaria to be the causal organism and identified it as *C. elvae*. Taylor and Baylis (151) examined similar material from the same lake a short time later and were able to produce a dermatitis in experimental subjects but identified the organism as *C. ocellata* La Val. St. George, 1855.

Szidat (147) reported a large number of cases from near Rossitten in East Prussia, in which he suspected the larva of *Bilharziella polonica*; but many experimental exposures of susceptible individuals failed to prove that this organism was a dermatitis provoker or could penetrate human skin. Vogel (153-155) observed a good many natural infections of what appeared to be cercarial dermatitis in bathers in the Holstein Lake region south of Kiel, Germany. He was successful in producing experimental infections in volunteers with *C. ocellata* from snails in the lakes where natural infections were known to have occurred. He made a careful study of the similarity of cercarial dermatitis in Germany and the skin eruptions in human beings reported by Nageli (109) from the Bodensee region in Switzerland.

It must be noted that none of these observers succeeded in identifying the adult phases of the parasites concerned. The work of Brumpt (17-19) in France, on material collected near Paris, not only confirmed the belief that *C. ocellata* was a dermatitis provoker but also that it was the larva of *Trichobilharzia ocellata* (Skrjabin and Zakharow, 1920) Brumpt, 1931. Price in 1931 (115) completed the life-history of *C. douthitti* Cort, 1916, which has since been found to provoke a dermatitis.

The etiology and epidemiology of cercarial dermatitis have been summarized in a number of articles the most valuable of which are by the following investigators: Fülleborn (58), Szidat and Wigand (148), La Rue (70), and Cort (38, 39). The disease was first reported from Canada by the writer in 1934 (92) and at that time it was thought to be confined to Clear Lake, Manitoba. However, information gathered personally during the past five years indicates that the causal organisms and their molluscan hosts are common in the majority of prairie lakes and sloughs in southern Manitoba (Fig. 2).

Dermatitis-provoking Cercariae

The number of species of larval trematodes that definitely bear the potentiality for provoking human dermatitis is uncertain at the present time. This is to be understood in view of the difficulty encountered in identifying species even by experienced workers. The larvae show very indefinite physiological characters and the tissues exhibit little differentiation. Cercariae from a single snail host show a fairly wide variation in size so that metromorphic characters are not to be relied upon entirely. No doubt, what are considered now as single species in a good many cases will be found later to represent a complex of several species.

Human susceptibility to the penetration of the organisms must also be taken into account. Apparently certain species of cercariae cannot penetrate the skins of all people and probably only a few species of those that do penetrate provoke a sensible skin reaction. This is particularly true of the schistosomes of man, concerning whose dermatitis-provoking powers there is a division of opinion.

A number of investigators have produced evidence both for and against the hypothesis that the cercariae of *S. haematobium*, *S. mansoni*, and *S. japonicum* are responsible for a dermatitis in man. A number of Japanese workers are of the opinion that "kabure", which resembles cercarial dermatitis in the United States, results from the attacks of the cercariae of *S. japonicum*. Miyagawa (100), however, maintains that this is not so, as "kabure" occurs in regions where schistosomiasis is not present and also that in endemic areas "kabure" does not result in infestations with adult *S. japonicum*. Faust and Meleney (55) state that a skin eruption does not occur among rice farmers in China where schistosomiasis is endemic, and Faust, Jones, and Hoffman (56) state that in Puerto Rico the cercariae of *S. mansoni* do produce a prickling sensation while penetrating the skin but a dermatitis does not result.

Vogel (155) found that the cercariae of *S. mansoni* produced a slight dermatitis on the arm of a white subject but not on two negroes exposed in the same manner. Manson-Bahr and Fairley (87) saw cases of dermatitis among Arabs in Egypt which definitely were due to attacks by the cercariae of *S. haematobium* and *S. mansoni*. Lee (71) saw a number of cases among gypsies in the Yangtse River area in China, and Bettencourt *et al.* (6) observed a case on the arm of a woman in Portugal. In each case the cercariae of human schistosomes were known to be present in the water with which the individuals had come in contact or were recovered from snails in the vicinity.

Fülleborn (58) is of the opinion that "kabure" results from the penetration of human skin by non-human schistosome cercariae. Cort (38, 39) is inclined to the view that the cercariae of the so-called human schistosomes seldom produce a significant dermatitis and that cercarial dermatitis is associated with the reaction of an abnormal host to the penetration of a non-specific cercaria. The writer is of the opinion that individual resistance and susceptibility are important factors as some exposures of human beings to non-human schistosome cercariae are negative, while positive cases vary greatly in extent and severity.

It is improbable that all the species of non-human schistosome cercariae can produce a dermatitis in man. The nature of the stimulus to which the organisms give a positive response is not well known and the morphological characters that determine certain aspects of their behaviour are poorly understood. It is not clear how many may be attracted to the human skin but be unable to penetrate it.

At present there are four general types of schistosome cercariae as regards tactic behaviour towards man:

1. Those that do not respond positively to the presence of a human being in the water.
2. Those that respond positively but are unable to penetrate the skin.
3. Those that respond positively and penetrate the skin but, owing to lack of balance between the host and parasite, do not advance deeply and thus provoke only a localized skin reaction.
4. Those that respond positively, penetrate the skin, and eventually make their way to the portal vein and its branches, where sexual maturity is reached. In this case it is thought that frequently no skin reaction occurs.

From the information available it can be assumed that only the cercariae of the eight schistosome species of man belong to group 4. A good many known species may belong to groups 1 or 2, but at the present time only eight species of non-human schistosome cercariae can be placed in group 3. Undoubtedly more will be added in the future, as a schistosome dermatitis is known in several localities where the causal organism has not been definitely identified, or erroneously identified as one of the eight so-called dermatitis provokers.

Those definitely known to provoke dermatitis are as follows:

Cercaria ocellata (La Val. St. George, 1855) Ssintzin, 1909.

C. douthitti Cort, 1916.

C. physellae Talbot, 1936.

C. elvae Miller, 1923.

C. pseudoocellata Svidat, 1933.

C. tuckerensis Miller, 1927.

C. dermolestes sp. nov.

C. stagnicolae Talbot, 1936.

In 1934 the writer incriminated two additional cercariae, both of which were described as new species. *C. bajkovi* McLeod, 1934, is an invalid species, as the characters of two new species were confounded and the description corresponds with neither one. Both species have since been described by Cort and Brackett (42, 43). Similarly the unnoticed production of small numbers of *C. stagnicolae* simultaneously with *C. wardlei* led the writer to assume that the latter was a dermatitis provoker. This work has been rechecked but requires additional confirmation one way or the other.

Furcocercous Cercariae of Manitoba

Seven species of longifurcate pharyngeal cercariae have been recognized in Manitoba, but repeated experiments have proved fairly conclusively that none of them is capable of provoking dermatitis in man. They are as follows:

Species	Manitoba host
Cercaria of <i>Diplostomum flexicaudum</i> Van Haitsma, 1931 (<i>C. flexicauda</i> Cort and Brooks, 1928)	<i>Lymnaea stagnalis jugularis</i> <i>Stagnicola emarginata canadensis</i>
Cercaria of <i>Cotylurus flabelliformis</i> Van Haitsma, 1931 (<i>C. douglasi</i> Cort, 1917)	<i>S. emarginata canadensis</i> <i>S. palustris elodes</i>

<i>C. yogena</i> Cort and Brackett, 1937	<i>S. emarginata canadensis</i>
	<i>S. palustris elodes</i>
<i>C. dohema</i> Cort and Brackett, 1937	<i>L. stagnalis jugularis</i>
	<i>S. emarginata canadensis</i>
Cercaria of <i>Crassiphiala ambloplitis</i> Hunter, 1937 (<i>C. bessiae</i> Cort and Brooks, 1928)	<i>Ilelisoma trivolvis</i>
<i>C. burti</i> Miller, 1923	<i>H. trivolvis</i>
<i>C. wardlei</i> McLeod, 1934	<i>S. emarginata canadensis</i>

In addition to the foregoing, three species of brevifurcate apharyngeal cercariae are common in Manitoba, and all three definitely are dermatitis provokers. They are as follows:

Species	Manitoba host
<i>C. elvae</i> Miller, 1923	<i>L. stagnalis jugularis</i>
<i>C. slugnicolae</i> Talbot, 1936	<i>S. emarginata canadensis</i>
<i>C. dermolestes</i> sp. nov.	<i>S. palustris elodes</i>

Descriptions of Cercariae

Cercaria dermolestes sp. nov.

SPECIFIC DIAGNOSIS: An apharyngeal brevifurcate cercaria with a prominent pair of pigmented eyespots. Entire body and tail uniformly spined. Body fusiform with the greatest diameter in the region of the acetabulum. Average measurements of specimens fixed in hot 10% formalin are as follows: Body 226μ in length by 70μ in maximum width; the anterior organ is 68μ in length and the acetabulum is 24μ in diameter and situated 94μ from the posterior end of the body. Tail stem 340μ by 42μ and furcae 210μ in length. Dorsoventral furcal fin folds are present. Five pairs of penetration glands are present, two being granular and circum-acetabular in position, while the remaining three pairs are post-acetabular in position. These open at the anterior end just lateral to the mouth on the oral sucker by means of fine ducts, the openings of which are located on fine spines. A pair of small escape glands is present in specimens dissected from the sporocyst. Digestive system consists of a tubular oesophagus, which branches in the region of the eyespots into two short blind caeca. The excretory system is composed of seven pairs of flame cells and their ducts. In each lateral half there are three anterior to the acetabulum, three posterior to the acetabulum, and one in the tail stem.

HOST: *Slugnicola palustris elodes* Say.

LOCALITY: Southern Manitoba, Canada.

If the above organism constitutes a legitimate species, this makes a total of four species of cercariae that are identical except for minor metromorphic differences, slight differences in behaviour, and also differences in the choice of snail hosts. They are as follows: *C. elvae* Miller, 1923, *C. slugnicolae* Talbot, 1936, *C. physellae* Talbot, 1936, and *C. dermolestes* sp. nov. The former three were considered as being conspecific for several years but were separated by Talbot in 1936.

All four have the same time of emergence from the snail host, namely 4.30 to 8.00 a.m. According to Talbot (150) and Cort and Talbot (41), *C. elvae* is positively phototactic and in the resting position attaches itself to the wall of the container and assumes a characteristic shape. The body is arched and the tail is bent at an acute angle over the back in the form of a hook with the furcae always crossed.

C. stagnicolae, according to the same authors, is always positively phototactic and hangs suspended from the surface of the water in the region of the greatest light intensity. In this case the body and tail stem are extended and rigid and the furcae are widely separated. They do not, however, attach themselves to the sides of the container.

C. dermolestes sp. nov. is morphologically almost identical with the three species described by Talbot (150) and is of approximately the same size but differs somewhat in relative body measurements. It most closely resembles *C. stagnicolae* Talbot, but the body is shorter, being 226μ on the average, and the tail stem is longer, being 340μ . It resembles *C. stagnicolae* in its behaviour but is a slow sluggish swimmer, and the resting periods are relatively short. At this time it hangs straight down in the water with the furcae only moderately diverged. It has less tendency to bend the body ventrad, so that frequently dorso-ventral mounts can be obtained.

To the writer's knowledge no other schistosome type of cercaria has been recorded from *Stagnicola palustris* except *C. douthilli* and, in view of the fact that at least six species of adult schistosomes occur in Manitoba, this might reasonably belong to a separate species. Larger numbers of this organism can penetrate a thicker barrier of clothing than either of the two other local species and the resulting dermatitis is much more pronounced. Thus, on the basis of morphological and physiological characters, host specificity, and pathogenicity, the writer is of the opinion that the above represents a new species and the name *Cercaria dermolestes* is suggested.

Cercaria wardlei McLeod, 1934 (Fig. 1; Plate I, 3)

This species was recorded from Clear Lake in 1934, but the description, while correct in all major points, is incorrect in one minor point and is not sufficiently detailed to be of any great value in identification. For this reason it is considered advisable to revise and extend the description.

SPECIFIC DIAGNOSIS: A large longifurcate pharyngeal cercaria with a stout body which is pointed at the anterior end, but the sides are almost parallel. Body measurements vary greatly between the largest and the smallest specimens, but the average measurements of a wide range of material fixed in hot 10% formalin are as follows: Body 194μ long by 45μ wide. The anterior organ measures 39μ in length, and the acetabulum which is 41μ in diameter has a distance of 95μ between its anterior margin and the anterior end. The tail stem is 298μ in length by 32μ in width and the furcae are 295μ in length. The oral region is surrounded by a dense band of about six rows of spines, and in the circum-oral spineless area dorsal to the mouth is located a group of

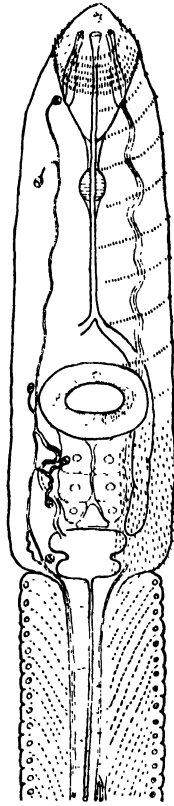


FIG. 1. Semidiagrammatic sketch of *Cercaria wardlei*; body and anterior portion of tail stem. About $\times 600$.

twelve anteriorly directed spines. The pre-acetabular region bears nine definite rings of spines and the post-acetabular region is uniformly spined. The tail stem bears about ten pairs of long lateral filaments. The pharynx is prominent and the caecal branches reach almost to the posterior end of the body. Three pairs of penetration glands are located posterior to the acetabulum and their ducts open lateral to the mouth. The excretory system is made up of eight pairs of flame cells and their tubules. In each lateral half, two of each anterior group of three are located in the pharyngeal region and the third lies lateral to the acetabulum. The posterior group of three are directed mesad and are fairly uniformly spaced. Two pairs are present in the tail stem and are equally spaced in the second and third fourths. Each lateral collecting tubule has a distinct convolution near its anterior end, and in each arm of this there is a patch of long motile filaments. The excretory bladder is large and has a constriction near its middle, at which point contractions take place during the constant pulsations of the bladder. Caudal bodies are absent, but there are lateral rows of about fifty musculo-epithelial cells in the tail stem. They have distinct nuclei, and the inner muscular portion shows distinct transverse

striations. Such cells are absent or much less prominent in other local cercariae and form an easy and reliable means of identifying *C. wardlei*. A similar type of cell has been reported from cercariae by Ssintzin (139).

C. wardlei is a powerful swimmer, but the swimming periods are short and the organism rotates as it passes along. When agitated it will swim back and forth for a considerable time before becoming quiescent. The resting periods are long and the organism hangs body downward from the water surface with the long furcae widely separated. The pre-acetabular portion of the body may be flexed ventrad or the tail stem may be flexed near its anterior end throwing the body either to right or left.

HOST: *Stagnicola emarginata canadensis*.

LOCALITY: Clear Lake, Manitoba.

Molluscan Hosts in Manitoba

Manitoba lakes and sloughs may be divided into three general ecological groups as regards geographic position, temperature, type of bottom, vegetation, and hydrogen ion concentration. The first, or what may be classed as the oligotrophic type, occurs in the eastern and northern part of the province, involving the Manitoba portion of the Pre-Cambrian shield and commonly known as the eastern region (2). Here the lakes are numerous and are usually very deep with rocky bottoms and very little vegetation. The temperature is low throughout the summer, the oxygen concentration is high, and the pH is neutral or moderately acid. Snails are relatively scarce, and cercarial dermatitis has never been reported from this region, so that it has not been included to any extent in the surveys.

The second or eutrophic type includes the vast majority of Manitoba waters of the prairie region, such as lakes, ponds, and sloughs. Here the water is shallow, somewhat turbid, and the summer temperature is relatively high. The inwash of material is large, the bottom is of loose organic ooze varying in depth and overlying more solid gravel or clay. The pH is on the alkaline side of neutrality. The essential difference between the prairie lake and slough is one of size and also of the amount of vegetation present in the littoral region owing to the differences in water currents.

In the typical prairie slough, plant zonation of the littoral region is fairly distinct. Farthest from the shore may be found such submerged or floating plants as filamentous and other algae, *Elodea* sp., *Polamogeton* sp., *Chara*, *Nitella*, and *Utricularia*. Toward the end of the warm season these may become very dense and reach to the water surface. Emergent vegetation near the shore usually shows more or less definite rings of *Typha*, *Scirpus*, and *Phragmites*. Among the dense vegetation there is usually an abundance of rotifers, nematode worms, crustaceans, snails, and larval insects.

Bays and protected bits of shore line of the larger bodies of water such as Lake Winnipeg, Lake Manitoba, and Lake Dauphin, in general, show much the same fauna and flora as the common slough. In many cases a definite

shore has not yet been formed in these recent lakes, and the body of water shades off into typical marsh or muskeg (Fig. 2; Nos. 11, 12, 14, 20, 21).

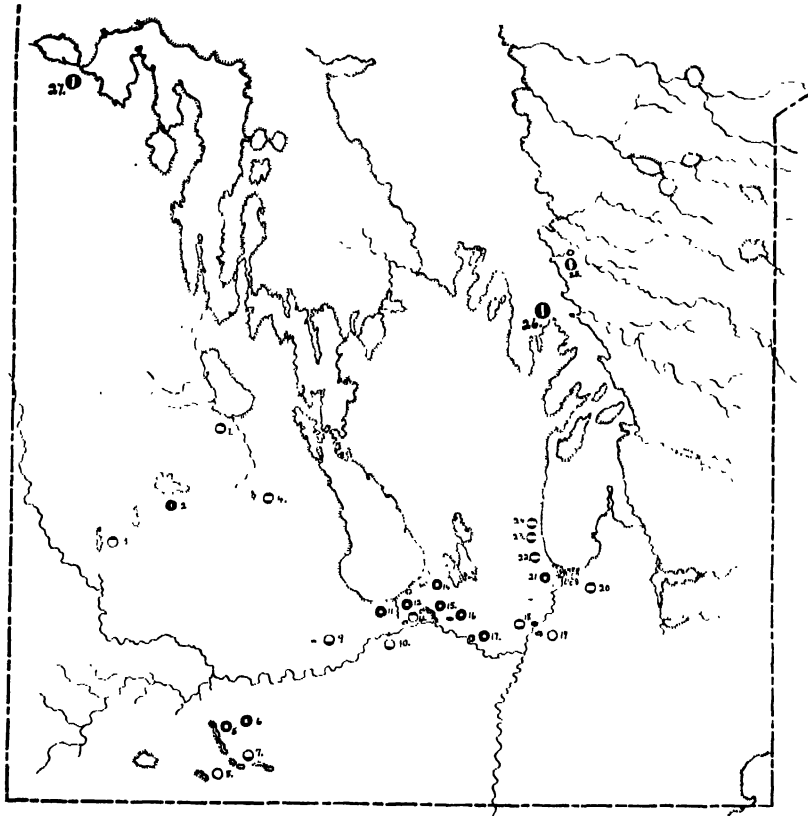


FIG. 2. Map of southern Manitoba showing districts studied. ● Cercarial dermatitis known to occur. — Molluscs found infested with schistosome cercariae and sporocysts. | Districts from which birds have been examined. | Districts from which birds were found infested with schistosomes. 1. Lake Dauphin, 2. Clear Lake, 3. Shoal Lake, 4. Gilmour Lake, 5. Pelican Lake, Ninette, 6. Pasture field slough near Ninette, 7. Lake Louise, Neelin, 8. Killarney Lake, 9. Artificial dam near Austin, 10. River lagoon, Portage la Prairie, 11. Delta marsh, 12. Portage Creek, 13. Black's Lake, High Bluff, 14. Lake Frances, 15. Long Lake, Raeburn, 16. Pasture field slough near Marquette, 17. Pigeon Lake, 18. River lagoon, St. Andrews, 19. Gravel pits, Birds Hill, 20. Libau marsh, 21. Neiley marsh, 22. Whitewold, 23. Ponemah, 24. Gimli, 25. Catfish Creek, 26. Blackbear Island, 27. Mafeking.

Clear Lake (Fig. 2; No. 2) represents a type intermediate between the oligotrophic and eutrophic lakes. It is situated at a relatively high altitude in an outcropping of the coniferous forest near the ecotone with the deciduous forest. There is little inwash of organic matter, as sizable inflowing streams are absent. The turbidity of the water is low and the currents are strong. The temperature is low, oxygen concentration high, and pH on the alkaline side of neutrality. The shore is rugged and cold water springs, either with or without definite channels, seep out of the bank in numerous places. The bottom near the shore is of fine sand and coarse stones or boulders. Emergent

vegetation such as reeds or rushes is sparse and confined to localized patches, but a growth of fine algae is found over the surface of the submerged stones.

Birds Hill area (Fig. 2; No. 19) represents an early stage of succession in rain and snow water pools in large gravel pits.

The molluscan fauna of Manitoba is moderately rich in species of Gastropoda, but many of these are few in numbers of individuals and are restricted in distribution (105, 106, 108). No doubt, in future more of these will be found to act as intermediate hosts to schistosomes, but only those that occur where cercarial dermatitis has been reported or suspected are included in the present paper. They are briefly as follows: *Lymnaea stagnalis jugularis* Say, *L. stagnalis perampla* Walker, *Stagnicola palustris elodes* Say, *Illeisoma trivolvis* Say, *II. campanulata* Say, *Physella gyrina* Say, *Fossaria obrussa* Say, *Valvata tricarinata* Say, *Amnicola* sp.

L. stagnalis jugularis is one of the largest and most widely distributed snails of Manitoba. It occurs in practically all the prairie lakes and permanent sloughs where the summer temperature is high and the vegetation dense. It was obtained from all the districts studied (Fig. 2) with the exception of 3, 8, 13, 19, and 25, and in every case many were found to be infested with the sporocysts and were liberating cercariae of the dermatitis-provoking type.

S. palustris elodes is a smaller but extremely abundant form found coincidentally with *L. stagnalis* in all districts except 9. In addition it was found in 19 and many other small or temporary bodies of water such as ditches, etc. Like *L. stagnalis*, wherever it occurs a sufficient distance from disturbance or human habitation, it is found to be the host to the larvae of schistosome and other types of trematodes.

S. emarginata canadensis, *L. stagnalis perampla*, and *F. obrussa* were found only at Clear Lake. *S. emarginata* is very abundant along the shore of almost the entire lake from a depth of 8 ft. to the water's edge. It is also the common host to the larval stages of a schistosome. Only three specimens of *L. stagnalis perampla* were found and these were uninfested. *F. obrussa* occurs in small numbers in the seepage water from springs around the edge of the lake. Only a few of these were examined, but all were free from trematode infestations. *II. campanulata*, *V. tricarinata*, and *Amnicola* sp. occur in the deeper waters of Clear Lake, but all three species were parasite free.

II. trivolvis and *Physella gyrina* occur in a wide range of situations but both were uninfested with schistosomes. On one occasion specimens of *II. trivolvis* from Pelican Lake were found to be infested with *C. burti* Miller and the cercariae of *Crassiphiala ambloplites* Hunter. An unidentified cercaria, possibly *C. multicellulata* Miller, was obtained on two occasions from *P. gyrina* at Clear Lake.

PRIMARY HOSTS IN MANITOBA

Many species of mammals are found in the vicinity of Manitoba lakes and sloughs and any one or more of these might reasonably act as the primary host of schistosomes. In recent years parasitological surveys of 460 Snowshoe

Rabbits (*Lepus americanus*) and 240 gophers (*Citellus*, 3 sp.) have been carried out in the laboratories of the University of Manitoba (12, 91) but all were negative for schistosomes. Other specimens examined included 36 muskrats (*Ondatra zibethica*) from Clear Lake and Netley and Delta marshes.

Representatives of the majority of Manitoba wild mammals have been examined in the laboratories of the Provincial Game and Fisheries Branch during the past eight years by Dr. J. A. Allan, Pathologist, but as yet no schistosomes have been found.

Five laboratory mice and three kittens were exposed to schistosome cercariae, but post-mortem examination one month later showed them to be free from blood trematodes.

Manitoba is visited annually by large numbers of water fowl either as migrants or summer residents. The ordinary prairie marsh or slough is the feeding and breeding ground of a good many ducks, coots, and grebes, as well as of a number of shore birds and waders. Gulls and terns are fairly common around the lakes and rivers, breeding in either neighbouring marshes or on rocky islands. Loons and merganser ducks nest around some of the deeper lakes but never occur in great numbers. Migrating cormorants, pelicans, and geese, as well as a number of others, remain for varying lengths of time in the spring and fall.

The fact that schistosome cercariae are widespread in Manitoba and that their occurrence coincides with that of the snail host except in certain small areas close to human habitations, indicated that the definitive hosts quite probably would be water birds. This condition, coupled with the fact that over half the known species of schistosomes have water birds as definitive hosts, led to the examination of all possible local species. However, insufficient numbers of some species were examined to give significant results.

Microbilharzia lari was recovered from Herring and Ring-billed Gulls collected at Lake Winnipeg and Clear Lake. Other specimens of Herring and Ring-billed Gulls from the same points as well as other places on Lake Winnipeg, Lake Winnipegosis, and Clear Lake yielded two additional species of schistosomes, namely: *Ornithobilharzia aviani* sp. nov. and *Ornithobilharzia filamenta* sp. nov.

Both species of gulls occur in considerable numbers around the larger Manitoba lakes and their tributaries. They also occur in moderate numbers at Clear Lake. A few loons and merganser ducks are found at Clear Lake, but ordinary pond or diving ducks are scarce during the breeding season or late summer. Blue-winged Teal and Golden-eye Ducks frequently alight and feed for a short time near the water's edge in the early morning.

The Blue-winged Teal is the commonest duck nesting around prairie lakes and sloughs. They are very numerous and widely distributed; over 60% of all specimens, both adult and juvenile, were infested with the males of *Pseudobilharziella querquedulae* McLeod, 1937. These were collected from a good many localities, and infested birds were encountered in every case regardless of the season.

TABLE I
BIRDS EXAMINED FOR SCHISTOSOMES

Specific name	Common name	No. examined	No. infested
<i>Larus pipixcan</i>	Franklin's Gull	7	0
<i>Larus argentatus</i>	Herring Gull	32	7
<i>Larus delawarensis</i>	Ring-billed Gull	20	5
<i>Sterna hirudo</i>	Common Tern	6	0
<i>Dafla acuta</i>	Pintail Duck	12	0
<i>Querquedula discors</i>	Blue-winged Teal	38	22
<i>Nettion carolinense</i>	Green-winged Teal	4	0
<i>Anas platyrhynchos</i>	Mallard Duck	9	0
<i>Nyroca valisneria</i>	Canvas-back Duck	3	1
<i>Spatula clypeata</i>	Spoonbill Duck	12	0
<i>Mareca americana</i>	Baldpate Duck	1	0
<i>Nyroca marila</i>	Bluebill Duck	3	0
<i>Nyroca americana</i>	Redhead Duck	30	0
<i>Nyroca collaris</i>	Ringneck Duck	1	0
<i>Erismatura jamaicensis</i>	Ruddy Duck	2	0
<i>Glaucionetta clangula</i>	Golden-eye Duck	1	0
<i>Branta canadensis</i>	Canada Goose	1	0
<i>Fulica americana</i>	Coot	7	0
<i>Colymbus nigricollis</i>	Eared Grebe	3	0
<i>Limosa fedon</i>	Marbled Godwit	1	0
<i>Phalacrocorax auritus</i>	Crested Cormorant	1	0
<i>Bartramia longicauda</i>	Upland Plover	1	0
<i>Gavia stellata</i>	Red-throated Loon	4	0
<i>Gavia immer</i>	Northern Loon	1	0
<i>Ceryle alcyon</i>	Belted Kingfisher	1	0
<i>Botaurus lentiginosus</i>	American Bittern	1	0
<i>Mergus americanus</i>	American Merganser	4	0
<i>Pelecanus erythrorhynchus</i>	White Pelican	1	0

Total number of species 28. Total number of specimens 207.

The Canvas-back Duck is common in southern Manitoba only during the seasonal migrations. A single specimen infested with *Microbilharzia manitobensis* McLeod, 1936, and *Microbilharzia canadensis* McLeod, 1936, was collected in late fall at Lake Frances.

An attempt was made to infect several species of wild ducks experimentally with each of the three species of schistosome cercariae. Hatchery-reared birds of from one to six weeks of age were obtained and some of each species were exposed repeatedly in the laboratory for intervals of from 15 to 45 min. to water containing the cercariae. In other cases thick infusions of cercariae were injected intravenously, intraperitoneally, or subcutaneously by means of a hypodermic needle.

Birds Exposed Experimentally

Specific name	Common name	Number
<i>Anas platyrhynchos</i>	Mallard Duck	5
<i>Dafla acuta</i>	Pintail Duck	5
<i>Nyroca valisneria</i>	Canvas-back Duck	5
<i>Nyroca americana</i>	Redhead Duck	4
<i>Querquedula discors</i>	Blue-winged Teal	10
<i>Anas boschas domestica</i>	Domestic Duck	6

Control birds were kept in each case in order to check the accuracy of the experiment. One domestic duck exposed to *C. stagnicolae* developed a severe dermatitis on the second day and died on the sixth day as a result. Post-mortem examinations were carried out up to six weeks after exposure but all were negative.

Classification of the Schistosomatidae

The discovery of several new species of schistosomes in the past few years, particularly those recorded by Ejsmont (47), Wetzel (158), and the writer (93, 94), necessitates a revision of the classification and also the keys for the identification of the various members of the family. In the following keys Price's classification (114) is used as a basis, but a number of minor changes are suggested.

Price (115, 116) suggests the re-establishment of the genus *Macrobilharzia* Travassos, 1923, to accommodate *Paraschistosomatium anhingae* Price, 1929, and *Ornithobilharzia macrobilharzia* (Travassos, 1923) Price, 1929, and suggests that the genus *Paraschistosomatium* Price, 1929, be deleted. This suggestion has been adopted in the following classification.

The genus *Ornithobilharzia* Odhner, 1912, should be amended, as suggested by Wetzel (158), to include forms in which the female is shorter or longer than the male, having 28 to 120 testes in the male, and also having the spiral ovary of the female confined to the anterior one-third of the body. This would necessitate the amending of the genus *Microbilharzia* as follows: Suckers present in the male, present or absent in the female. Testes 18 to 26 in number in the male, ovary pre-equatorial in the female and in the form of a loose spiral. The species *Ornithobilharzia lari* McLeod, 1937, would then be transferred to the genus *Microbilharzia*. The creation of the new genus *Pseudobilharziella* by Ejsmont (48) calls for a revision of the generic diagnosis of the old genus *Bilharziella*, and he suggests the following changes: Anterior region narrow and one-half of the body length; posterior region broad and lanceolate. Genus *Pseudobilharziella*: Anterior portion of the body broad and one-fifth of the body length; posterior portion narrow and strap-like.

A modification of Price's classification in accordance with the above suggestions consequently would be as follows:

KEY TO THE SUB-FAMILIES OF THE SCHISTOSOMATIDAE

Females slender, more or less cylindrical in cross-section; males larger than females, flattened, and with the lateral edges infolded forming a gynaecophoric canal; intestinal caeca usually unite caudad of the equator of the body; testes situated cephalad of the caecal union. . . . SCHISTOSOMINAE.

Females similar to males in shape; males without well developed gynaecophoric canal; caecal branches unite cephalad of the equator of the body; testes situated caudad of the caecal union. BILHARZIELLINEAE.

KEY TO THE GENERA OF SCHISTOSOMINAE Stiles and Hassal, 1898

1. Females unknown; male gynaecophoric canal well developed; testes 70 to 83 in number, in posterior third of body and cephalad of the caecal union; in mammals. *Heterobilharzia*.
Males and females both known. 2.
2. Testes 230 to 250 in number, from just caudad of acetabulum into posterior half of body; female slender, much shorter than male; ovary spiral, in the posterior third of the body; intestinal caeca unite in posterior end of body; uterus long and filled with eggs. *Macrobilharzia*.
Gynaecophoric canal well developed; testes less than 120. 3.
3. Testes 28 to 120 in number, in the anterior two-thirds of the body; female shorter or longer than male; ovary spiral in anterior one-third of body. *Ornithobilharzia*.
Testes 26 or less in number; ovary just pre- or post-equatorial in position; suckers present in male, present or absent in female. 4.
4. Anterior end of gynaecophoric canal near equator of body; testes in two rows, at anterior end of gynaecophoric canal; genital pore immediately in front of anterior testis; intestinal caeca with short lateral diverticula; common caecum in both sexes short; ovary pre-equatorial. *Schistosomatium*.
Anterior end of gynaecophoric canal near acetabulum; genital pore of male a short distance caudad of acetabulum; intestinal caeca with diverticula reduced or absent; common caecum usually long; ovary pre- or post-equatorial in position. 5.
5. Testes less than ten in number; ovary oval. *Schistosoma*.
Testes 18 to 26 in number; ovary spiral. 6.
6. Anterior end of gynaecophoric canal slightly caudad of acetabulum; oral sucker lacking in female; ovary about one-third of body length from the posterior extremity. *Austrobilharzia*.
Anterior end of gynaecophoric canal cephalad of acetabulum; suckers present or absent in female; ovary pre-equatorial. *Microbilharzia*.

KEY TO THE GENERA OF BILHARZIELLINAE Price, 1929

1. Body cylindrical or nearly so. 2.
Body flattened. 3.
2. Female unknown; posterior end of body threadlike, middle portion wider than either the anterior or posterior portions; no gynaecophoric canal; suckers present. *Trichobilharzia*.
Male and female very long and slender; gynaecophoric canal reduced to a short groove in the anterior part of the body; suckers absent. *Gigantobilharzia*.
3. Suckers absent; common caecum with short lateral dendritic branches. *Dendritobilharzia*.
Suckers present; common caecum without lateral dendritic branches. . . . 4.

4. Body divided into narrow anterior and broad lanceolate posterior portions, the point of union being at the junction of the second and third fifths of the body.....*Bilharziella*.
 Body divided into broad anterior and narrow strap-like posterior portions, the point of union being at the junction of the first and second fourths of the body.....*Pseudobilharziella*.

KEY TO THE SPECIES OF THE GENUS MICROBILHARZIA Price, 1929

1. Testes in male reaching into post-equatorial region.....2.
 Testes in male confined to pre-equatorial region.....3.
 2. Body fairly stout in male; caecal branches slightly convoluted and without diverticula; female unknown.....*M. chapini*.
 Body slender in male; caecal branches convoluted and with small diverticula; female unknown.....*M. manitobensis*.
 3. Caecal branches in male uniting at about the junction of the middle and posterior thirds of body; common caecum long; acetabulum wanting in female.....*M. canadensis*.
 Caecal branches in male uniting near hinder end of body; common caecum short; acetabulum present in female.....*M. lari*.

ADULT SCHISTOSOMES OCCURRING IN MANITOBA

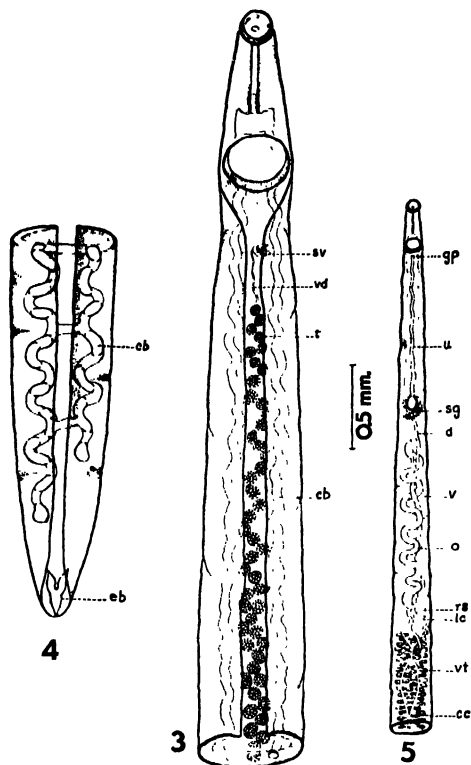
Species	Host
<i>Microbilharzia canadensis</i> McLeod, 1936.	<i>Nyroca valisneria</i> .
<i>M. manitobensis</i> McLeod, 1936.	<i>N. valisneria</i> .
<i>M. lari</i> McLeod, 1937.	<i>Larus argentatus</i> .
<i>Pseudobilharziella querquedulae</i> McL. 1937.	<i>Querquedula discors</i> .
<i>Ornithobilharzia aviani</i> sp. nov.	<i>L. argentatus</i> .
<i>O. filamenta</i> sp. nov.	<i>L. delawarensis</i> .
	<i>L. argentatus</i> .

Descriptions of New Species

Ornithobilharzia aviani sp. nov. (Figs. 3-5)

GENERIC DIAGNOSIS: *Ornithobilharzia*.

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter, reaching a maximum of 0.83 mm. The lateral edges of the body are infolded to form a well developed gynaecophoric canal which begins just caudad of the acetabulum and gradually becomes more pronounced. It is only moderately deep throughout most of its length and gradually disappears near the caudal end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed oblique ventrad. It is circular and has an average diameter of about 250 μ . The anterior body portion is elliptical in



FIGS. 3-5. *Ornithobilharzia aviani* sp. nov. FIG. 3. Anterior portion of male. FIG. 4. Posterior end of male. FIG. 5. Anterior portion of female. cb. caecal branch; cc. common caecum; d. vitelline duct; eb. excretory bladder; gp. genital pore; lc. Laurer's canal; o. ovary; rs. receptaculum seminis; sg. shell gland; sv. seminal vesicle; t. testes; u. uterus; v. vagina; vd. vas deferens; vt. vitellaria.

cross-section and the acetabulum is pedunculate. Its anterior margin is 0.82 mm. from the anterior end of the body and the average diameter is 400μ . The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back, it passes through a mass of tissue, partly muscular, partly glandular in nature. Cephalad of the acetabulum, it passes into a squarish cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a slightly convoluted course caudad. Farther back, the caeca become progressively larger and more convoluted and later, anastomosis occurs. There are some individual variations near the posterior end where there may be one or two anastomosing branches. Following this, the caeca may be of unequal length and end singly or may unite, the common caecum ending close to the posterior end of the body. A small but distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in a row, which at the anterior end is convoluted, while toward the hinder part it is made up of a compact mass about two testes in width. The row begins about 0.68 mm. caudad of the acetabulum and extends into the post-equatorial region. In mature speci-

mens, the testes are spherical, about 95μ in diameter, and vary from 54 to 74 in number. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. caudad of the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Filamentous worms considerably shorter than the males and usually completely enclosed in the gynaecophoric canal. The average length in fixed specimens is 7.2 mm. by 0.23 mm. in width. The portion anterior to the ovary is cylindrical and finely attenuated, while the hinder portion is flattened and bluntly pointed. The cuticle is finely tuberculate. The oral sucker is terminal and has an average diameter of 50μ . The acetabulum is well developed and pedunculate, with a diameter of 63μ . The digestive canal branches into two fine caeca just cephalad of the acetabulum. These pass caudad to just behind the ovary where they may unite permanently or separate and reunite once or twice. The common caecum follows a straight course to near the hinder end of the body. The ovary is a spiral consisting of about eight loops and begins 1 mm. from the acetabulum. Its length, disregarding the total length of the spiral, is 0.78 mm. A small receptaculum seminis is present at the posterior end of the ovary and Laurer's canal is also present. An oviduct of moderate size passes forward to the ootype, which is distinct and located 0.81 mm. caudad of the acetabulum. The vitellaria are in the form of numerous small oval follicles, which occupy most of the region caudad of the ovary. The vitelline duct passes forward in company with the oviduct to the ootype. The uterus is narrow, moderately straight and the genital pore opens behind the acetabulum. Eggs are produced singly.

CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, birds (*Larus argentatus*), secondary, unknown.

TYPE LOCALITY: Lake Winnipeg, Manitoba, Canada.

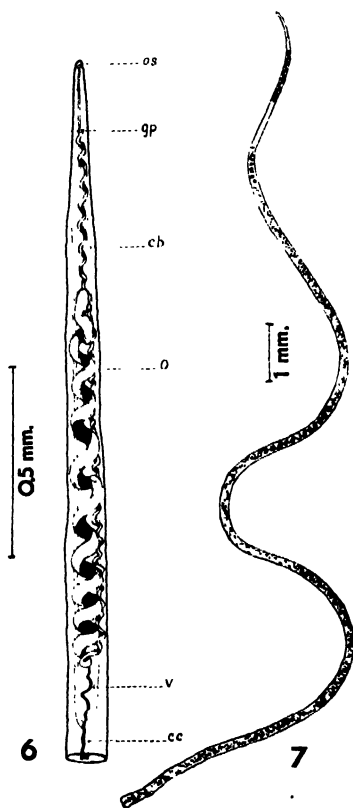
LOCATION: Portal and intestinal veins.

The above description is based on the examination of a few pairs of males and females in a good state of preservation, taken in copula from the intestinal veins of an adult specimen of *Larus argentatus*. Both males and females closely resemble those of *O. canaliculata* (Rudolphi, 1819) Odhner, 1912. The male also resembles that of *O. kowalewskii* (Parona and Ariola, 1896) Odhner, 1912, in so far as the brief descriptions of these two species go. Dr. Price suggests that there is a strong possibility of the two being conspecific, but because of the brevity of the published descriptions, it is impossible to establish the point. The writer considers it advisable to regard the above as a new species until such time as specimens of both *O. canaliculata* and *O. kowalewskii* can be examined in detail and the differences of similarities determined for all three.

Ornithobilharzia filamenta sp. nov. (Figs. 6 and 7)

GENERIC DIAGNOSIS: *Ornithobilharzia*.

MALE: Large straight-bodied worms of an average length in fixed specimens of 12 to 15 mm. and of a fairly uniform diameter reaching a maximum of 0.63 mm. The lateral edges of the body from just behind the acetabulum to very near the posterior end are infolded to form a fairly well developed gynaecophoric canal. It is only moderately deep in the mid-body region and becomes



FIGS. 6 and 7. *Ornithobilharzia filamenta* sp. nov. FIG. 6. Anterior portion of female. FIG. 7. Entire female. cb. caecal branch; cc. common caecum; gp. genital pore; o. ovary; os. oral sucker; v. vitelline duct.

progressively shallower toward the posterior end. The edges normally do not meet in the mid-line. The cuticle is thick and without tubercles or spines. The oral sucker is terminal and in the form of a shallow funnel, the aperture of which is directed obliquely ventrad. It has a length of about $210\ \mu$ and a width of about $205\ \mu$. The anterior portion of the body is elliptical in cross-section and the acetabulum is pedunculate. Its anterior margin is about 0.81 mm. from the anterior end and the average diameter is $330\ \mu$. The mouth is situated in the centre of the oral sucker and leads into a narrow straight tube. A short distance back it passes through a mass of dense,

deeply staining tissue and anterior to the acetabulum it passes into a bulbous cavity of considerable size. The caecal branches arise from the posterior-lateral margins of this and follow a convoluted course caudad. Toward the posterior end, they become progressively larger with traces of secondary branches and show a tendency to anastomose. In certain cases they unite to form a common caecum, but in others they unite by cross branches and each ends blindly. A small distinct Y-shaped excretory bladder opens at the posterior tip of the body. The testes are arranged in an irregular single row, which follows a zig-zag course. The row begins about 0.7 mm. behind the acetabulum and extends to within about 4.4 mm. of the posterior end. In mature specimens the testes are oval, $89\ \mu$ in diameter, and vary in number from 88 to 112 in different individuals. The vas deferens is narrow and moderately straight. It opens into a transversely elongated seminal vesicle. The genital pore is considerably to the left of the median line and is about 0.28 mm. behind the acetabulum. A prostate or cirrus pouch is absent.

FEMALE: Extremely long and filamentous, with a length of 15 to 20 mm. and a maximum width of 0.1 mm. A short anterior portion of the body is cylindrical and finely attenuated, while the remainder is broader with parallel sides, flattened, and ends bluntly. The oral sucker is feebly developed and is $16\ \mu$ wide. It is conical in shape and the aperture is subterminal in position. An acetabulum is entirely lacking and the cuticle is finely spined. The ovary is an elongated tubular body forming a spiral of about 8 to 9 turns and lies in the middle of the anterior one-eighth of the body, beginning about 0.61 mm. from the anterior end. The vitellaria are in the form of a single row of numerous relatively large follicles, each of which is almost as wide as the body. They occupy the posterior seven-eighths of the body. The oviduct passes forward from the posterior end of the ovary, and the uterus appears to open a short distance from the anterior end. The ootype and Mehlis' gland were not discernible and eggs were not present in the female worms. The alimentary canal is simple, branching midway between the oral sucker and the ovary, and the caecal branches reunite just posterior to the ovary. The common caecum is long, reaching almost to the posterior end and following a zig-zag course.

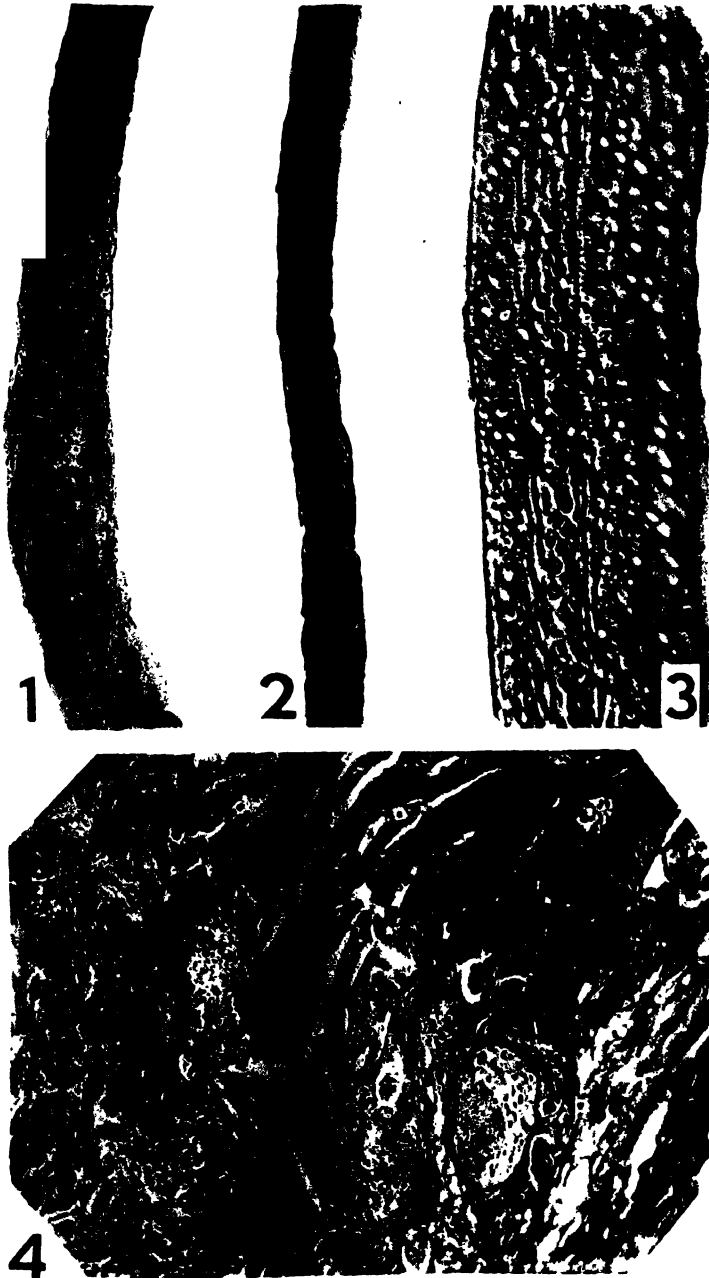
CERCARIA: Unknown or unrecognized.

TYPE HOST: Primary, Birds (*Larus delawarensis* and *L. argentatus*); secondary unknown.

LOCALITY: Lake Winnipeg and Clear Lake, Manitoba, Canada.

LOCATION: Coccyeomesenteric and mesenteric veins and lymphatics.

The male of *Ornithobilharzia filamenta* is almost identical morphologically with that of *O. aviani*, except for the number of testes present. The two species occur coincidently in the same host, so for this reason, only males actually in copula with the females have been used in determining the specific characters. The female differs greatly in size and also in the shape and position of the genitalia from that of *O. aviani*, and thus serves to distinguish the two



1. Photomicrograph of genital region of female *Ornithobilharzia aviani* sp. nov., showing spiral ovary, seminal receptacle, caecal union, oviduct, shell gland and vitellaria, $\times 60$.
2. Photomicrograph of mid-body region of female *Ornithobilharzia filamenta* sp. nov. showing single row of large vitellaria, $\times 60$.
3. Photomicrograph showing tail stem musculature of *Cercaria wardlei*, $\times 940$.
4. Photomicrograph of section of lymphatic from infested Herring Gull showing sections of female *Ornithobilharzia filamenta* sp. nov. and fibrosed cyst containing what appears to be eggs, $\times 20$.

species at a glance. *O. filamenta* has been found in the hepatic portal and mesenteric veins but appears to prefer the coccygeomesenteric vein, as females frequently are found there in large numbers. In some cases they are found to have migrated into the neighbouring lymphatics and produced granular, tumour-like swellings of considerable size. Histological sections of one of these tumours (Plate I, 4) show sections of several female worms and also what is taken to be groups of eggs that have been walled off by fibrous tissue. Lesions of the gut wall have not been found and free eggs have not been seen.

The males of the above described species, as in *O. aviani*, are very similar to those of *O. canaliculata* and *O. kowalewskii*. The female, in general, resembles that of *O. intermedia* and that of *O. pricei* but is much longer, the ovary is relatively farther forward, and the acetabulum is lacking entirely. The characters of the gynacophoric canal and testes in the male and the ovary and vitellaria in the female definitely place the above organism in the genus *Ornithobilharzia*. It differs, as pointed out, particularly in the case of the female, from any described species and is submitted as being new to science. The name *Ornithobilharzia filamenta* is suggested.

Discussion

A total of 15 species of adult schistosomes has been recorded from the United States and Canada. One of these (*B. polonica*) had been described previously from other countries and the life-history worked out in detail, but the cercariae have not been reported from North America. Two of the 14 species considered as being native to North America have their adult stages in mammals (rodents), and in both species the life-history is known and the cercariae have been described and their distribution studied. The remaining 12 have been reported as adults from birds and the cercarial stages are either unknown or unrecognized. Whether the infestations are acquired in these two countries or in some other is, as yet, undetermined. Six distinct species of schistosome cercariae are thought to occur in North America, and no doubt several more will be discovered in the near future or are being mistaken at the present time for one or more of the recorded species.

Six species of adult schistosomes have been found in Canada by the writer, and all these occur in migratory birds in the southern half of Manitoba. Two species are rare, having been found only once in the Canvas-back Duck, a bird that occurs here only as a migrant.

Pseudobilharziella querquedulae is the commonest schistosome found locally. It occurs in 60% of all the specimens of *Querquedula discors* examined, regardless of the season or the place from which the birds were collected. *P. querquedulae*, undoubtedly, is the adult of one of the three local dermatitis-provoking cercariae, as immature worms have been recovered from fledgling birds that were not yet able to fly but had frequented waters of prairie lakes, where both *Cercaria elvae* and *C. dermolestes* were abundant.

It is remarkable that no females of this species have been found in so large a number of examinations in which males were plentiful. There is the

possibility that they have been overlooked on account of their small size. A more logical explanation, perhaps, is that *Q. discors* is an unnatural host and only the male schistosomes develop in it. This was found to be the case by Faust (53) in infections of human schistosomes in unnatural hosts such as the rabbit and sheep.

Microbilharzia lari and *Ornithobilharzia aviani* are found in local adult Herring Gulls in fairly large numbers. It is not known where the infection is contracted, there being no more evidence to favour the winter feeding ground than the summer habitat. *Diplostomum flexicaudum* is carried in the adult stage by the Herring Gull, and the larvae occur coincidentally with schistosome cercariae at Clear Lake. This might indicate that *C. stagnicolae* is the larva of one of the gull schistosomes. Immature male worms of both species were obtained from birds collected in late autumn and, as the time required for maturation in most schistosomes is usually three months or less, this would strengthen the theory that the infection occurred in local waters.

O. filamenta was found in both Herring and Ring-billed Gulls from Clear Lake in midsummer and from Lake Winnipeg in midsummer as well as late autumn. Mature and immature males and females were recovered and the above argument would apply equally to these.

Consistent failure to obtain adult worms by exposure of experimental animals may be due to a number of causes. In addition to the experimental work by the writer, Talbot (150) exposed young domestic ducklings, Herring Gulls, and a pigeon to three species of schistosome cercariae from Douglas Lake, Michigan, but the results were negative in every case. Differences in the temperature, the pH, or the salt concentration of the water used in the laboratory may have been responsible for the production of cercariae so inviable that they were unable to penetrate the skin of experimental animals, or these changes may have interfered with their normal responses to the presence of a host, although this seems unlikely.

Effective treatment of cercarial dermatitis once it has been contracted by human beings is very difficult. The intense itching and annoyance are the most serious aspects, and treatment is usually directed to relieve these. Application of various lotions or ointments is usually recommended, but the writer has found them to be of little or no value in either reducing the irritation or killing the organisms.

The direct pathological effects of schistosomes on the bird host is not known in any detail, but infested birds are invariably lighter and more emaciated than uninfested ones. Much additional work is required on the life-histories and pathogenicity of the local forms.

Acknowledgments

This problem was carried out under the direction of Prof. R. A. Wardle, to whom the writer gives his sincerest thanks for constant support, valuable advice, and kind assistance. A large number of people have given inform-

ation, sent in material, or assisted in other ways, and to those people grateful appreciation is expressed. The writer would particularly acknowledge the kindness of the late Dr. D. A. Stewart, and Mr. David Stewart, Ninette, Man., Mr. Edward Ward, Delta, Man., Mr. A. G. Cunningham and Mr. David Hinks, Provincial Game and Fisheries Branch, Dr. W. W. Cort, Johns Hopkins University, Dr. W. A. Riley, University of Minnesota, and Prof. A. H. R. Buller, University of Manitoba.

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THE HELMINTH PARASITES AND PARASITIC DISEASES OF SHEEP IN CANADA

I. A SURVEY AND SOME PRELIMINARY STUDIES ON EXISTING PROBLEMS¹

BY W. E. SWALES²

Abstract

Investigations have been made upon the identity, incidence, economic importance, and seasonal fluctuations of the helminth parasites in the alimentary canal of sheep in Canada, special consideration having been given to the problems in Quebec and eastern Ontario. Haemonchiasis is the parasitic disease of major importance in Eastern Canada during the summer months, and is replaced by trichostrongylosis, oesophagostomiasis, and possibly hookworm disease in the late autumn and winter. Haemonchiasis and chabertiasis were recorded in British Columbia.

Introduction

Sheep husbandry has not flourished in Canada. At the present time there are no more sheep in the Dominion than there were in 1871, and at that time the important sheep ranges in the Prairie Provinces were not being used. The number of sheep in Ontario in 1937 was 874,769, compared with 1,915,303 in 1882. The decrease in Quebec over the same period was not as great, and the number has approximated 750,000 for the last ten years.

The lack of expansion and actual decrease in sheep production in Eastern Canada have been due to a number of causes, chief among which are probably the difficulty of accommodating and feeding breeding stock during the winter months, a period of low prices obtained for meat and wool, high costs of fencing materials, lack of protection against marauding dogs and predatory animals, increased need of available pastures near cities for dairy cattle, and disease. However, in many outlying areas in Eastern Canada large tracts of land are unsuitable for the production of dairy products but could be adapted for sheep production on a small unit basis, and would produce this "cash crop" with a minimum of expenditure or labour. In those areas the problems of fencing and winter accommodation could be overcome by the present availability of cheap labour and lumber. Shipping costs are now comparatively low, predators are fewer in numbers and prices for good market animals have steadily improved. Canada has been fortunate in having a very low incidence, and in many cases complete absence, of such important bacterial, virus, and protozoal diseases of sheep as anthrax, foot and mouth disease, louping ill, necrobacillosis, variola ovina, heart-water, piroplasmosis, scrapie, and braxy. Psoroptic mange has not been allowed to become established and calliphorine

¹ Manuscript received June 28, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada. Presented, in part, before the Third International Association of Microbiologists, New York, September, 1939.

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myiases are comparatively uncommon. Haemorrhagic septicaemia must be considered as an important problem, although some preventive measures can be applied during periods of exposure. Contagious ecthyma appears to be of moderate importance in range flocks, but is unlikely to become prevalent under eastern conditions.

Diseases due to internal parasites appear to be major causes of losses, particularly in Eastern Canada. Owing to climatic extremes, species that are normally distributed in temperate climates and some that are usually considered as being important only under semi-tropical conditions, are enabled to complete their life cycles on pastures in the Eastern Provinces. It is thought by competent authorities that parasitic disease has been and is still the greatest deterrent to profitable production of sheep in many parts of the Dominion. Extension work has reduced losses considerably in some agricultural districts, particularly in Ontario; the methods of control generally employed have been those in use in the United States of America, and have been directed chiefly against verminous gastritis in lambs due to infestations of *Haemonchus contortus*. However, discouraging losses and lack of thriftiness in lambs and breeding stock have continued throughout many areas, particularly in Eastern Canada.

In the hope that a thorough investigation of the incidence, economic importance, and control of the parasites of sheep in Canada would contribute towards more successful sheep husbandry and would encourage farmers to extend this industry, a series of studies was commenced in 1936. The present paper represents the first of the series, and includes briefly the results of the preliminary work.

The Occurrence of Species

The following species have been found in sheep and lambs in Canada, in natural infections, during the course of these studies.

<i>Parasite</i>	<i>Estimated Prevalence</i>
CLASS—TREMATODA	
<i>Dicrocoelium dendriticum</i>	Recorded in Nova Scotia only.
<i>Fasciola hepatica</i>	Confined to small areas.
CLASS—CESTODA	
<i>Moniezia expansa</i>	Very common.
<i>Thysanosoma actinioides</i>	Common in Western Canada.
<i>Cysticercus tenuicollis</i>	Very common.
<i>C. ovis</i>	Rare.
CLASS—NEMATODA	
<i>Ascaris</i> sp.	Rare.
<i>Strongyloides papillosus</i>	Very common.
<i>Oesophagostomum columbianum</i>	Very common in Eastern Canada.
<i>O. venulosum</i>	Common in British Columbia.
<i>Chabertia ovina</i>	Very common.
<i>Monodontus trigonocephalus</i>	Very common.
<i>Dictyocaulus filaria</i>	Not common.
<i>Muellerius capillaris</i>	Very common.
<i>Trichostrongylus axei</i>	Very common.

<i>Parasite</i>	<i>Estimated Prevalence</i>
CLASS—NEMATODA—conc.	
<i>T. vitrinus</i>	Very common.
<i>T. colubriformis</i>	Very common.
<i>Cooperia curticei</i>	Very common.
<i>C. oncophora</i>	Not common.
<i>Ostertagia circumcincta</i>	Very common.
<i>Haemonchus contortus</i>	Very common.
<i>Nematodirus filicollis</i>	Very common.
<i>N. spathiger</i>	Common.
<i>Gongylonema pulchrum</i>	Rare.
<i>Trichuris ovis</i>	Very common.
<i>Capillaria longipes</i>	Not common.

The above list includes only two additions to other published records in Canada (Swales (12) and Fallis (5)), these being *Gongylonema pulchrum* and *Capillaria longipes*. It is, therefore, considered to be an accurate list of all species that are liable to be encountered in reasonably large samples. The expected discovery of other species of *Trichostrongylus* and *Ostertagia* has not been made.

Special Technique Employed in Autopsies

In estimating the numbers of individuals of the larger species, *Haemonchus contortus*, *Moniezia* spp., *Monodontus trigonocephalus*, *Trichuris ovis*, *Chabertia ovina*, and *Oesophagostomum columbianum*, direct counts of all individuals screened from intestinal washings were made. In light infections *Ostertagia circumcincta* and *Nematodirus* spp. were also counted individually. In order to estimate the numbers of the small species, *Trichostrongylus* spp., *Cooperia* spp., and *Strongyloides papillosus*, it was necessary to employ a dilution technique.

Owing to the importance of making accurate estimations the examination of a small portion of a formalin suspension of the intestinal contents was not found adequate. After numerous tests a simple technique was evolved which has proved to be a reliable and rapid method, and which has been adopted as the standard technique in these studies. This method is described in some detail owing to the interest expressed in it by several visiting parasitologists.

After removal from the animal the abomasum is ligated at the omaso-abomasal orifice and at the pylorus, the duodenum being left attached to the abomasum. The ileum is ligated at or near the ileo-caecal valve. The whole is then immersed in 5 to 10% formalin in a tank large enough to conform to the rules necessary for adequate fixation; specimens are left in this solution for at least two days.

The preserved abomasum is examined by opening it over a pail, and a stream of water is run on to the mucous membrane as it is thoroughly rubbed with the fingers. The suspension of ingesta and parasites is then screened through a stack of sieves (Fisher's U.S. Standard Sieve Series Nos. 10, 20, and 40) which have mesh diameters or nominal openings of 2.00 mm., 0.84 mm., and 0.42 mm. respectively, and with wire diameters of 0.76 mm., 0.42 mm., and

0.25 mm., and which are 21.7 cm. in over-all diameter. The type of sieve as devised by Hall and Cram is not suitable for this method.

As the sieves are filled, a well-fitting cover is placed over them and they are shaken with a rotary motion. Water is added until most of the soluble material is removed. Each sieve is now inverted in a large moist chamber dish made of thick glass and the contents are washed off with water which has been cleared of air bubbles. The contents of each dish are allowed to settle and the supernatant water is then decanted. The large species are now counted, the dish being placed over a black surface and under a strong light, for this purpose. The contents of the dish are then transferred to another dish which has a diameter of 25 cm. and a bottom area of 491.0 sq. cm. The contents, which should be approximately half an inch in depth, are gently shaken until they are evenly distributed in the dish; a metal ring is quickly placed in the mass in such a way that its edge is near the centre of the dish. This ring, which has an area of 49.1 sq. cm. thus separates one-tenth of the contents of the dish, and this amount is pipetted into a square Petri dish which has been ruled to fit the field of a dissecting microscope. The ring used in this work is the screw-top of a one-pint glass preserving jar, the bottom edge having been levelled and sharpened.

The one-tenth sample from each dish is then examined systematically under a magnification of $\times 25$ for the detection and identification of the genera of small parasites.

In 20 tests with 1,000 adult *Ostertagia circumcincta*, this sampling technique indicated the number within 10% in 19 tests, the mean error being $\pm 4.45\%$.

This technique requires some practice and experience in spreading the ingesta in the dish and placing the ring, in order to obtain accurate results, but its use approximately doubled the number of specimens that it was previously possible to record by the use of other reasonably accurate methods.

Observations on the Abundance and Pathogenicity of Parasites in Lambs in Eastern Canada

In 1936 a preliminary survey of the parasites in 134 abomasa of market lambs was made. The lambs came from farms in the southern sections of Quebec and in eastern Ontario. The collections were made at random in abattoirs in Montreal. The results of the examinations of the contents, made under laboratory conditions, are shown in Table I.

The low numbers of individuals recovered from these animals would be due, in part at least, to the fact that the lambs were relatively healthy specimens and were slaughtered between October 23 and November 25.

As noted by Fallis (5) and as illustrated later in this paper, the peak of abundance of *H. contortus* occurs in the summer and then the number of individuals decreases rapidly in the months of autumn. *O. circumcincta* has a similar fluctuation, but *T. axei* occurs in greatest numbers during the late autumn or winter months.

TABLE 1
ABOMASAL PARASITES OF 134 LAMBS FROM QUEBEC AND EASTERN ONTARIO

Parasite	No. of lambs infected	Percentage infected	Mean no. of worms per infected lamb	Min. and max. nos. of worms
<i>Haemonchus contortus</i>	82	61.2	45.4	1 — 530
<i>Ostertagia circumcincta</i>	99	73.9	154.2	3 — 1,620
<i>Trichostrongylus axei</i>	124	92.5	605.4	20 — 8,710

The results of these preliminary observations on abomasal parasites agree in all important details with those of Fallis in Ontario, seasonal variations being considered, and for this reason no further details are included.

The degree of infection with certain species of nematodes which is able to cause clinical disease or at least mark unthriftiness in lambs or adult sheep, has been the subject of some study. An examination of the literature reveals that some workers have set a tentative line between tolerated and harmful infections for known pathogenic species. It is usually considered that mixed infections are more harmful than infections with a single species, and this supposition, supported by some evidence of lowered resistance to one species being produced by another, will remain for the present a complication in preliminary observations on pathogenicity. Ross and Gordon (10) state that it is highly improbable that infections with less than 500 *H. contortus* are ever the primary cause of serious effects, and that fatal cases are seldom seen with less than 1,000 individuals of this species. They point out the importance of the complicating factor of nutrition in such estimation.

Robertson (9) estimates that fatal results are not produced by less than 8,000 individuals of *O. circumcincta*. Taylor's (14) estimations of numbers of this species would indicate that fatal results are produced by this species, but that about 16,000 individuals are necessary.

Trichostrongylus spp. are the undoubted cause of severe gastro-enteritis in Great Britain, Africa, and Australia, and under suitable climatic conditions must always be considered as potential pathogens. Ross and Gordon state that fatal infections are seldom induced in Australia by less than 10,000 of these worms in the abomasum and small intestine, and state at the same time that Taylor's work indicates that in Great Britain the number is considerably larger.

Experimental evidence that *Cooperia curticei* in very heavy infections (10,000 to 25,000 individuals) affects the economy of lamb production has been brought forward by some workers, notably Andrews (1).

There is a lack of experimental evidence of the effect of the parasites of the large intestine, but it is generally agreed that heavy infections with *Chabertia ovina* or *Oesophagostomum columbianum* are very harmful. Ross considers that more than 100 individuals of either of these parasites constitute a heavy infection.

For the purpose of comparisons for the preliminary work, the above findings and opinions were used for the adoption of tentative standards for conditions in Canada. Thus, infections which were considered harmful are:—

<i>Haemonchus contortus</i>	over 500 individuals
<i>Ostertagia circumcincta</i>	over 8,000 individuals
<i>Trichostrongylus</i> spp.	over 10,000 individuals
<i>Cooperia curticei</i>	over 10,000 individuals
<i>Chabertia ovina</i>	over 100 individuals
<i>Oesophagostomum columbianum</i>	over 100 individuals

In comparison with the above mentioned nematodes, all of which are commonly encountered in Canada, the other common parasites, *Moniezia expansa*, *Thysanosoma actinioides*, *Nematodirus* spp., *Strongyloides papillosus*, *Trichuris ovis* and *Muellerius capillaris*, were not considered as of primary importance but were always considered in subsequent observations. *Monodontus trigonocephalus* could not be placed in either of these groups owing to lack of data on its pathogenicity.

A large number of very poor lambs from the Eastern Provinces are placed on the market each year. From time to time viscera from such animals were obtained from abattoirs in order to collect specimens for identification, and in most cases a large number of nematodes or lesions due to the invasion of *Oesophagostomum columbianum* were present. It was thought that most of these animals were suffering from the effects of severe helminthiasis, although it was realized that observations made upon such animals would be of limited value without knowledge regarding other conditions, particularly those of nutrition, to which they had been subjected. However, in order to collect data on the limits of infection in these and in healthy stock from the same districts a number of observations were made. Lambs only were studied, and these were divided into "Cull lambs" which were thin and under 30 lb. dressed weight, and "Market lambs," which were healthy in appearance and over 30 lb. dressed weight. In the unhealthy lambs the dressed weight was, in most cases, somewhat less than 45% of the live weight, and in the healthy lambs it was usually slightly more than 50%.

The results of the examinations of these lambs are presented in Tables II, III, and IV. Each table represents animals killed during the same month, so that seasonal fluctuation of species need not be considered in the comparisons within each table.

It will be noted that the significant difference between the "Cull" and "Market" lambs in Table II (August) is in the number of *Haemonchus contortus* only. In Tables III and IV (September and October) this significant difference is not present, nor is there any other significant difference. This may be explained by the seasonal fluctuation of *H. contortus*, and would indicate that if any one species of parasite was responsible for the condition of the "Culls" it was *H. contortus*, the pathological effects having been produced earlier in the season.

TABLE II
LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE.
Killed in August, 1938

Lambs	<i>Haemonchus</i>	<i>Ostertagia</i>	<i>T. axei</i>	<i>Moniesia</i>	<i>Mono-</i> <i>dontus</i>	<i>Nematodirus</i> spp.	<i>Cooperia</i> spp.	<i>Tricho-</i> <i>strongylus</i> spp.	<i>S.</i> <i>papillosus</i>	<i>T. onis</i>	<i>Chaberita</i>	<i>Oso-</i> <i>phago-</i> <i>stomus</i>	Nodules
"Calf"													
1	103	60	230	0	5	220	260	430	0	40	0	1	22
2	285	9,550	900	7	8	146	310	230	1,060	4	0	20	53
3	90	230	260	0	5	108	280	300	40	53	1	0	6
4	1,295	1,020	540	1	37	432	1,660	1,060	80	31	0	11	175
5	177	67	90	4	11	138	290	80	70	104	0	0	54
6	4,134	4,880	520	5	105	317	3,790	1,050	40	19	26	0	71
7	185	147	150	0	2	590	0	180	760	27	0	0	5
8	2,284	920	60	7	10	1,313	50	40	20	8	0	0	14
9	143	10,970	450	7	36	50	320	600	160	2	0	28	316
10	3,547	165	100	0	18	83	140	0	480	10	1	0	14
Totals	12,243	28,009	3,300	31	237	3,397	5,600	3,970	2,710	298	28	60	730
Means	1224.3	2800.9	330	3.1	23.7	339.7	560	397	271	29.8	2.8	6	73
"Market"													
1	19	940	360	0	73	22	90	50	140	89	0	7	138
2	3	780	540	3	16	15	320	120	60	9	0	7	184
3	241	330	80	8	12	26	0	2,770	0	2	5	0	117
4	30	303	80	0	4	258	30	280	40	2	6	0	8
5	188	621	0	0	47	36	0	80	30	0	0	0	1
6	21	334	80	0	3	66	40	30	20	22	0	0	6
7	57	1,172	630	14	23	324	10	260	70	27	7	0	13
8	23	60	160	0	6	116	50	250	400	2	1	0	11
9	20	566	0	0	7	59	40	140	0	7	2	0	0
10	651	1,470	460	90	4	1,210	270	900	0	17	11	1	32
11	280	410	100	16	21	349	0	250	50	16	1	0	6
12	3	10	10	0	0	0	0	0	0	1	0	1	40
13	21	30	10	2	2	19	0	0	0	5	1	0	23
14	494	2,020	0	0	0	1,280	300	320	0	4	0	0	0

TABLE II—*Concluded*

LAMBS FROM LOTS SHIPPED FROM LUCEVILLE, BLACK LAKE, TROIS PISTOLES, NAPIERVILLE, STE. CHRISTINE, AND POINTE FORTUNE, QUE.
Killed in August, 1938—*Concluded*

Lambs	<i>Haemonchus</i>	<i>Ostertagia</i>	<i>T. axei</i>	<i>Moniezia</i>	<i>Mono-</i> <i>donius</i>	<i>Nematodirus</i> app.	<i>Cooperia</i> app.	<i>Tricho-</i> <i>strongylus</i> app.	<i>S.</i> <i>papillosus</i>	<i>T. ovis</i>	<i>Chabertia</i>	<i>Oesophago-</i> <i>stomum</i>	Nodules
"Market" —con.													
15	462	500	30	0	0	60	20	50	0	6	0	0	7
16	469	230	90	0	0	30	0	100	0	12	1	1	10
17	269	261	160	2	2	23	10	30	20	9	0	1	34
18	157	237	0	0	0	1,070	0	0	0	39	0	0	2
19	10	123	120	0	0	8	0	0	0	9	0	2	49
20	138	3,010	4,360	16	0	30	120	2,180	0	5	0	8	147
21	76	34	0	3	0	15	0	40	30	2	0	0	37
22	980	340	30	0	0	32	20	10	20	2	0	0	13
23	45	780	350	0	13	32	30	90	250	8	0	0	14
Totals	4,657	14,561	7,650	154	233	5,080	1,350	7,950	1,130	295	35	28	892
Means	202.5	633.1	332.6	6.7	10.1	220.9	58.7	345.6	49.1	12.8	1.5	1.2	38.8

SAMPLE TESTS FOR SIGNIFICANT DIFFERENCES

Lambs	Parasite	Means	S.D.	Mean diff. \pm S.E. diff.	P
"Cull"	<i>Haemonchus</i>	1224.3	1555.1	1020.9 \pm 498.6	0.05
"Market"	<i>Haemonchus</i>	203.4	397.2		
"Cull"	<i>Ostertagia</i>	2800.9	4203.2	2167.5 \pm 1331.0	0.1
"Market"	<i>Ostertagia</i>	633.4	354.6		

TABLE III
LAMBS FROM BEAUCE COUNTY AND MANIWAKI
Killed in September, 1938

Lambs	<i>Haemonchus</i>	<i>Ostertagia</i>	<i>T. axei</i>	<i>Moniezia</i>	<i>Monodontus</i>	<i>Nematodirus</i> spp.	<i>Cooperia</i> spp.	<i>Trichostrongylus</i> spp.	<i>S. papillosus</i>	<i>T. onis</i>	<i>Chabertia</i>	<i>Oesophagostomum</i>	Nodules
"Cull"													
1	188	1,100	0	0	63	34	20	50	450	6	25	1	4
2	21	2,200	460	12	11	320	160	4,950	0	51	14	0	61
3	428	100	60	5	35	20	200	120	20	1	16	6	116
4	2,014	940	290	18	134	42	1,150	3,180	450	3	3	32	34
5	1,276	1,350	940	35	52	24	90	1,590	80	5	5	6	454
Totals	3,927	5,690	1,750	70	295	439	1,620	9,890	1,000	66	63	45	669
Means	785.4	1,138	350	14	59	88	324	1,998	200	13.2	12.6	9	133.8
"Market"													
1	0	0	170	0	6	10	0	190	0	2	2	3	275
2	140	1,020	50	89	54	571	190	730	230	4	16	66	195
3	167	1,100	620	16	21	31	90	600	160	28	7	54	321
4	1,349	1,390	170	0	8	5	510	230	0	33	3	4	196
5	26	1,500	300	9	25	169	310	420	310	30	9	29	309
6	82	70	370	0	60	40	370	560	80	0	21	5	177
Totals	1,764	5,080	1,680	114	174	826	1,470	2,730	780	97	58	161	1,473
Means	294	846.6	280	19	29	137.7	245	455	130	16.1	9.7	26.8	245.5

TABLE IV
LAMBS FROM FRONTENAC COUNTY, QUE. (FOUR FLOCKS, CONTROLS FOR EXPERIMENTAL TREATMENTS)
Killed in October, 1938

Lambs	<i>Haemonchus</i>	<i>Ostertagia</i>	<i>T. axei</i>	<i>Moniezia</i>	<i>Monodontus</i>	<i>Nematodirus</i> spp.	<i>Cooperia</i> spp.	<i>Trichostrongylus</i> spp.	<i>S. papillorum</i>	<i>T. ovis</i>	<i>Chabertia</i>	Oesophagostomum	Nodules
"Cell"													
1	1,414	2,650	4,190	7	53	75	10,950	4,060	280	10	5	25	1,613
2	130	1,500	2,630	12	32	60	2,720	3,790	220	1	0	2	1,299
3	138	610	1,820	5	99	430	4,330	810	230	1	1	3	641
4	240	890	1,440	10	8	30	3,600	2,380	60	11	0	2	1,561
5	226	1,920	3,190	10	43	60	3,310	720	200	11	0	24	1,630
6	130	950	3,990	6	28	210	1,780	350	80	9	0	5	748
7	186	660	1,280	5	57	30	1,310	530	220	1	0	5	726
Totals	2,464	9,180	18,540	55	320	895	28,000	12,640	1,290	44	6	66	8,218
Means	352	1311.3	2648.6	7.9	45.7	126.4	4,000	1805.7	184.3	6.3	0.9	9.4	1,174
"Market"													
1	140	730	2,600	6	16	20	1,030	2,290	60	4	0	4	1,431
2	109	270	1,340	12	33	310	3,340	1,160	240	13	0	6	1,040
3	152	560	1,870	12	32	10	1,840	1,250	110	9	1	2	1,379
4	792	1,370	4,380	9	175	0	850	1,720	440	11	2	19	930
5	105	510	3,340	0	33	38	900	2,710	50	33	0	5	1,098
6	68	270	5,970	19	17	30	2,140	1,760	680	12	1	2	1,003
7	40	80	4,190	0	5	0	210	1,440	0	11	1	1	1,169
8	110	250	3,180	3	25	20	80	440	0	13	1	0	50
9	727	260	430	10	5	0	50	560	70	44	0	0	154
Totals	2,243	4,300	27,300	71	341	428	10,440	13,330	1,650	150	6	39	7,254
Means	249.2	477.7	3033.3	8	38	47.6	1,160	1481.1	183.3	16.6	0.66	4.3	806

In order to support the above hypothesis, and for purposes of comparison with data obtained by Fallis (5) and other authors on seasonal fluctuation, further observations were made upon the incidence of the various species in a single flock. All the lambs from this flock were slaughtered as good market lambs, and the observations were made over a period of two seasons and thus on two crops of lambs from the same stock and on the same pasture. The data are presented graphically, each graph showing the observed fluctuations in the mean number of individuals of a species from July to March (Figs. 1-11). The number of animals observed was 275, being distributed as follows:—

July	27	October	54	January	14
August	33	November	24	February	15
September	93	December	8	March	7

Although the observations in some months were, of necessity, small, these graphs serve to illustrate the tendencies of fluctuations more accurately than data from mixed lots of lambs from widely separated districts. Such variations as the fall in the number of *Nematodirus* spp. in August, for example, cannot be accepted as true deviations from a curve, but the gross contours of the curves appear to illustrate the usual tendencies in eastern Ontario and Quebec, and agree closely with those compiled by Fallis in southern and western Ontario.

NOTES ON PARASITIC DISEASES OF LAMBS IN LATE AUTUMN AND WINTER IN EASTERN CANADA

From the above data one would not expect to find verminous gastritis due to *H. contortus* or *O. circumcincta* in lambs in Eastern Canada during the late autumn or winter months. Nevertheless a large number of animals, from 7 to 11 months of age, are marketed each year during the cold months in extremely poor condition. Some are so emaciated that they weigh less than 20 lb. dressed, and are frequently held under observation by Federal Inspectors for mucoid degeneration. Table V shows the results of necropsies on sample specimens of these emaciated lambs, which were all less than 20 lb. dressed weight. These findings indicate two features of particular interest, the superinfection of *Trichostrongylus* spp. in three animals, the heavy infection of *Monodontus* in three, and the very large number of lesions due to *Oesophagostomum columbianum* in all the animals. These lesions, when present in large numbers, literally cover the intestines of small animals, frequently extending from the abomasum to the rectum. In very heavy infections there is little doubt regarding the pathogenic effect of the causative agent, particularly in those numerous cases in which partial or complete occlusion of the ileo-caecal valve has been produced. The number of lesions on the small intestines is approximately the same as on the large intestines, but the region around the ileo-caecal valve appears to be constantly filled with nodules.

Owing to the fact that the effects of infection by *Oesophagostomum columbianum* in lambs in Eastern Canada appear to differ in several important details from those in other parts of the world, this disease has been the subject

of special studies, the results of which will be reported in a later paper. As the position of the lesions on the intestinal tract appears to exert a greater influence on the health of the host than mere numbers, a tentative line between tolerated and harmful infections is difficult to set. It is apparent, however, that less than 300 lesions do not affect the normal development of the young animal, and that under certain conditions a well grown lamb can tolerate a much larger number.

The Parasites of Adult Stock in Eastern Canada

Because of the methods of marketing sheep in Canada, whereby the young stock is sold as "lamb" from July until the following spring at from 4 to 11 months of age, it is difficult to obtain viscera from relatively young breeding stock for parasitological studies. These marketing practices are due to economic necessity, because the cost and labour of overwintering any sheep other than those to be used for breeding could not be recovered from future sales. Thus a suitable market for "mutton" has not been developed, and adult sheep are usually marketed only when they have become unprofitable to their owners and must be sacrificed at a low price. Sheep which appear on the markets in the spring, early summer and in the winter are frequently in poor condition, and the majority are more than three years old.

Adult sheep in Eastern Canada are almost invariably infected with lesions due to *O. columbianum*; during the course of these studies only two lots of sheep from the Province of Quebec that included individuals free from this infection have been seen. These lots came from Lac St. Jean and Amqui, two more northerly points of agricultural Quebec; all other lots have shown numerous lesions in all individuals.

During the course of field investigations, small numbers of ewes have been killed and examined on farms in attempts to determine the cause of emaciation and inability to nourish their lambs. Severe infection with lesions of oesophagostomiasis have been the only constant pathological process noted. Samples of emaciated ewes placed on the market in Montreal during the spring and early summer have been examined. The number is too small for any definite decision regarding the cause of the emaciation, but, in the absence of any obvious pulmonary infection, the findings in regard to helminthic infections are of some interest. The results of necropsies on eight ewes over four years of age are shown in Table VI. These findings appear to indicate the probable effect of the numerous lesions of oesophagostomiasis in the presence of only light infections of other helminth parasites in the intestinal tracts.

The Parasites of Sheep and Lambs from Western Canada

Large numbers of lambs from the ranges in southern Saskatchewan and Alberta are slaughtered in Eastern Canadian abattoirs each year. These animals are usually excellent fat lambs of the Rambouillet breed. The most striking feature noted during the abattoir operations is that these animals are entirely free from lesions of oesophagostomiasis. There is, however, a

TABLE VI
EMACIATED ADULT EWES FROM EASTERN CANADA

—	<i>Haemon- chus</i>	<i>Oster- tagia</i>	<i>T. axei</i>	<i>Moniezia</i>	<i>Mono- dontus</i>	<i>Nemato- dirus</i> spp.	<i>Cooperia</i> spp.	<i>Tricho- strongy- lus</i> spp.	<i>S. papil- losus</i>	<i>T. ovis</i>	<i>Chaber- tia</i>	<i>Oeso- phago- stomum</i>	Nodular lesions
1	0	4	97	0	13	0	12	8	?	0	0	3	1,180
2	0	16	50	0	8	10	40	110	?	0	0	10	1,088
3	2	10	70	0	8	0	0	20	?	0	0	0	520
4	0	10	310	0	5	10	0	30	?	0	0	0	1,685
5	0	7,900	4,720	0	45	50	0	210	?	0	1	6	887
6	0	1,750	2,850	0	52	150	70	300	?	0	69	48	760
7	194	460	950	0	12	0	170	10	?	0	0	17	543
8	10	150	0	0	23	0	340	0	40	0	0	0	1,099

fairly high incidence of infection in the intestine and bile ducts with the Fringed Tapeworm, *Thysanosoma actinioides*. A sample of 18 animals from a "feed-lot" in western Ontario, which had been received previously from southern Saskatchewan, was examined in 1934. All these animals were infected with *T. actinioides*; although tapeworms were present only in the bile ducts of five livers, the majority were in the small intestines. Very light infections of both *H. contortus* and *O. circumcincta* were present in all the abomasa, but the greatest number of these stomach worms in a single lamb was 30. Fourteen of the small intestines contained small numbers of *Nematodirus filicollis*, 12 contained small numbers of *Trichostrongylus colubriformis*, and six contained up to ten *Monodontus trigonocephalus*. All the large intestines contained small numbers of *Trichuris ovis* and two contained up to five *Chabertia ovina*. In addition to the above, one specimen of *Moniezia expansa* and two of *Capillaria longipes* were recovered from the lot.

The only infection of interest in these fat lambs was that of *T. actinioides*; 100 of these parasites were present in one small intestine and two were in the liver of the same animal.

During these studies the abattoir operations on several hundreds of fat western lambs have also been observed and a high incidence of *T. actinioides* in the bile ducts has been noted. These observations have led the writer to agree with Christiansen (3) that this parasite is not of major importance.

Viscera from western sheep and lambs that were reputed to be suffering from parasitic disease have been examined from time to time. The results of these examinations are shown in Table VII, the numbers of parasites in all but the last four animals being approximate.

The animals from Saskatchewan and Alberta were examined for the purpose of routine diagnosis and the numbers of individual parasites were not accurately recorded. However, in no case was an infection of pathological significance noted. The cases from British Columbia are very interesting. The animal from Agassiz was pastured on land which is subject to a very high summer rainfall and relatively high summer temperatures, and was undoubtedly suffering from haemonchiasis. In addition, the number of hookworms present was of probable clinical significance.

The three lambs from Vavenby had been subjected to entirely different conditions, and had ranged over land in the drier interior of the province. They were samples of a number of animals that had suffered from an enteritis characterized by scouring, with dark fetid faeces. The diagnosis in these three cases was severe chabertiasis, extensive damage having been produced in the crown of the small colon. These cases are of particular interest because they constitute definite evidence of the occurrence of a parasitic disease in a part of the Dominion that is of major importance in sheep husbandry. The number of individuals of *Chabertia ovina* present in each animal appears to be greater than any cases recorded from other countries.

TABLE VII
PARASITES IN THE INTESTINAL TRACTS OF UNHEALTHY SHEEP AND LAMBS IN WESTERN CANADA

Animal	Origin	<i>Haemon- chus</i>	<i>Ostertagia</i>	<i>T. axei</i>	<i>Moniezia</i>	<i>Thysan- osoma</i>	<i>Mono- donius</i>	<i>Nemato- dius</i> sp.	<i>Cooperia</i> sp.	<i>Tricho- strongylus</i> sp.	<i>S. papillans</i>	<i>T. onis</i>	<i>Chaber- sia</i>
Lamb	Edmonton, Alta.	0	50	10	0	2	0	1,539	40	0	0	3	0
Lamb	Edmonton, Alta.	0	410	0	0	0	0	1,149	30	150	0	0	0
Lamb	Tilly, Alta.	50	400	?	1	0	0	Few	Few	Few	?	5	0
Lamb	Tilly, Alta.	200	2,000	?	Few	0	0	700	12	?	?	12	7
Lamb	Cypress Hills	150	75	?	0	0	0	30	?	?	?	0	0
Lamb	Hatton, Sask.	0	Few	?	3	0	0	130	?	Few	?	0	0
Lamb	Piapot, Sask.	10	Few	?	10	0	0	40	?	?	?	1	0
Ewe	Maple Creek, Saak.	40	14	?	1	0	0	40	?	?	?	0	0
Ewe	Agassiz, B.C.	1,487	4,880	520	5	0	105	317	3,790	1,050	40	19	26
Lamb	Vavenby, B.C.	0	1,750	830	0	0	0	?	?	?	?	30	610
Lamb	Vavenby, B.C.	0	7,950	2,390	4	0	0	2,080	800	600	?	7	682
Lamb	Vavenby, B.C.	0	3,420	3,510	3	6	0	2,500	3,010	3,190	?	75	658

Discussion

In these preliminary studies an attempt has been made to show which species of parasitic worms are commonly present in sheep and which are of major importance. Data have also been presented in order to explain causes of unthriftiness in groups of animals, chiefly from Eastern Canada. From these data it is apparent that verminous gastritis, due to infection with *Haemonchus contortus*, is an important disease of lambs during the grazing season in the Province of Quebec and probably in all parts of Eastern Canada.

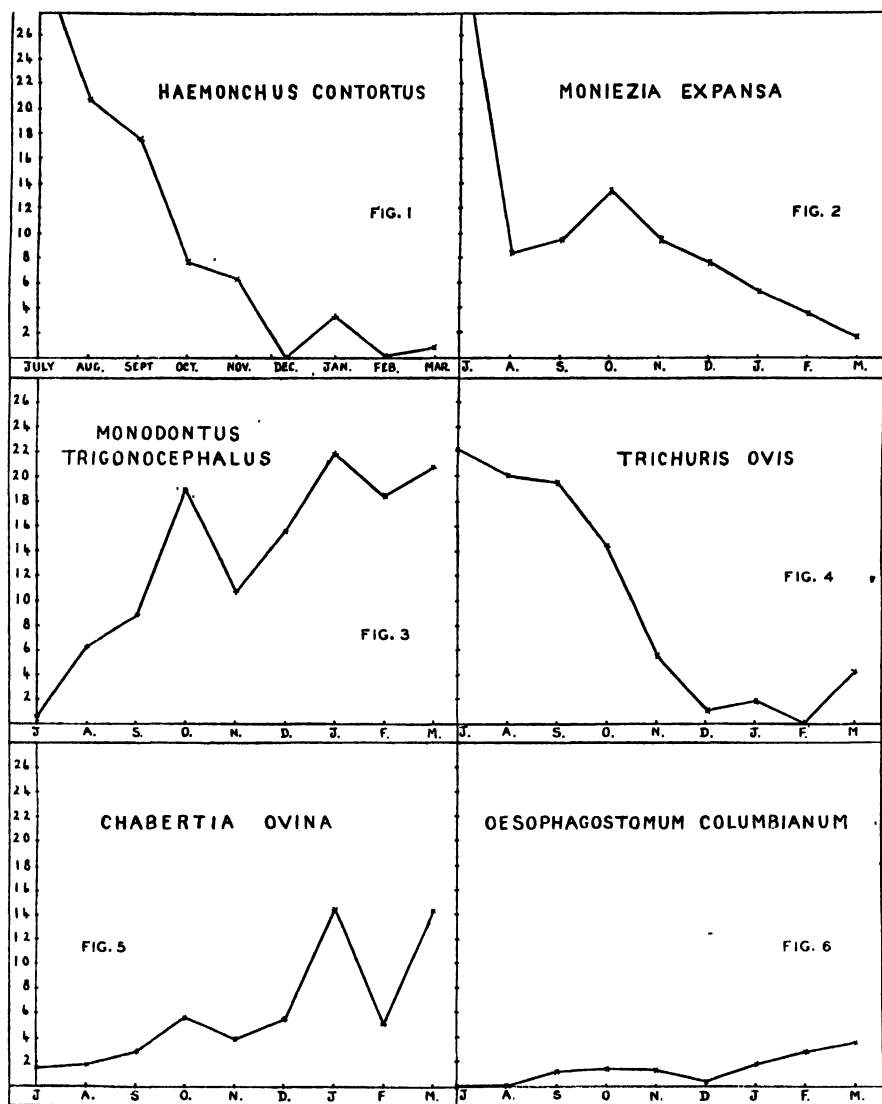
Infection with *Ostertagia circumcincta* has not been found of major importance, but, having reference to findings in Great Britain and elsewhere, this paper has not shown that this parasite cannot become of importance under certain conditions during the summer.

In the autumn and winter months in Eastern Canada verminous gastritis becomes of lesser importance, but heavy infections of *Trichostrongylus* spp. and the lesions due to invasion of the intestinal walls by larvae of *Oesophagostomum columbianum* become the apparent causes of unthriftiness.

In adult sheep in Eastern Canada the lesions of oesophagostomiasis appear to be a cause of marked unthriftiness during the breeding season.

The nature of parasitic disease in sheep in the Prairie Provinces has not been made apparent. In British Columbia records of importance have been made; in one region which is subject to a high summer rainfall, haemonchiasis and possible hookworm disease were recorded, and in a drier region in the interior of the province diagnoses of severe chabertiasis were made.

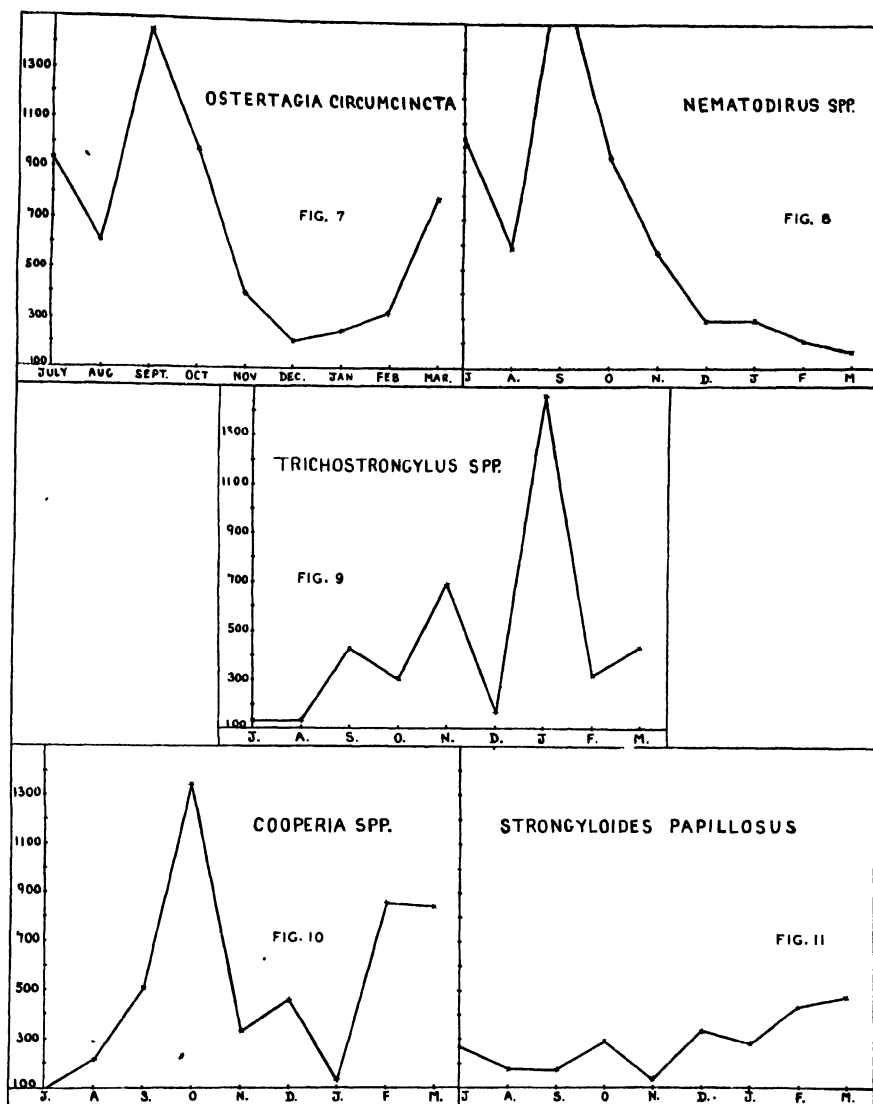
These preliminary surveys were made necessary by the fact that certain species of parasites that are of known pathogenic importance in one region may be present but of little or no importance in another region which is subjected to a different climate and different methods of husbandry. The pastures in Eastern Canada are not used during the winter months, when they remain under snow for a long period. Griffiths (6) has shown that *Moniezia expansa*, *Ostertagia circumcincta*, *Nematodirus filicollis* and *Trichostrongylus colubri-formis* are able to survive a normal winter on a pasture in the Province of Quebec. If the free-living stages of a certain species do survive and are present on the pastures in the spring, then one might expect this species to be present in reasonable numbers in lambs after they have grazed on the pasture for from six to eight weeks. Figs. 1 to 11 show the relative incidence of the common species in July, and would indicate, according to the above hypothesis, that *M. expansa*, *T. ovis*, *O. circumcincta*, *Nematodirus* spp. and *H. contortus* survived the winter. Accordingly, then, it is also apparent that winter survival in the case of *M. trigonocephalus*, *C. ovina*, *O. columbianum*, *Trichostrongylus* spp. and *Cooperia* spp. is low or non-existent. It will be noted, however, that *H. contortus* was present only in abnormally small numbers in these animals, and is numerically comparable to five other species that are usually present in small numbers (compare with data obtained from



FIGS. 1-6. The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.

market animals). Owing to this abnormality, which is probably due to the fact that the flock was treated with anthelmintics known to have a positive action upon *H. contortus*, this species cannot be considered in the hypothesis regarding winter survival.

The factor of non-survival on the pastures, combined with factors of summer rainfall and temperature, would have a profound effect upon the ability of certain species to be of pathogenic importance. These preliminary studies have, then, demonstrated the importance of knowledge regarding the biology



FIGS. 7-11. The seasonal fluctuation in numbers of the species of helminth parasites in the alimentary canal of lambs in Eastern Canada.

of the free-living stages of each species of helminth parasite present in sheep in Canada.

It is already apparent that owners of sheep in Eastern Canada must adopt efficient means of combating haemonchiasis in their animals during the summer months, and that they have urgent need of some measures of control of oesophagostomiasis in order to protect the health of breeding stock. The possible occurrence of disease due to heavy infections of *Trichostrongylus* spp., *M. trigenocephalus*, and *C. ovina* must always be considered.

Acknowledgments

Work on this subject could not be conducted without the co-operation of many individuals and organizations. The author acknowledges the excellent co-operation of the officials and employees of Canada Packers Limited, both at Montreal and Hull, Que., and of Wilsil's Ltd., Montreal. The Inspectors of the Department of Agriculture stationed at these plants have always offered invaluable help, particularly Inspectors Tanner, Labelle, Wood, and Young-husband. Mr. A. A. MacMillan, Dominion Department of Agriculture, has been a continual source of encouragement. Mr. W. Hicks, Agassiz, B.C., and Mr. T. K. Moilliet, Vavenby, B.C., submitted valuable material. Thanks are also due to Dr. J. E. Lattimer, Macdonald College, for advice regarding the economics of sheep husbandry in Canada.

The Quebec Department of Agriculture has been of material assistance, and Dr. J. M. Veilleux, Chief of the Health of Animals Service, Quebec, has co-operated in field tests of control measures.

Messrs. P. E. Sylvestre and S. Williams, Division of Animal Husbandry, Central Experimental Farm, Ottawa, have been active co-operators in tests of anthelmintic medication, the results of which will be published under joint authorship in this series.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

FEBRUARY, 1940

NUMBER 2

THE INTERRELATION OF LIPIDS IN THE BLOOD PLASMA OF WHITE LEGHORN COCKERELS¹

BY ELDON M. BOYD² AND ELEANOR L. CLARKE³

Abstract

The blood plasma of 22 healthy, young, white leghorn cockerels was analysed by oxidative micromethods and found to contain the following mean lipid values, in milligrams per 100 ml. of plasma: total lipid 520, neutral fat 225, total fatty acids 361, total cholesterol 100, ester cholesterol 66, free cholesterol 34, phospholipid 155. In general, the amounts of the various lipids were proportionately related to the total lipid.

In recent communications, Boyd has shown that when values for the various lipids of blood plasma of man (3) and rabbits (4) are plotted against increasing values for total lipid, it is demonstrated that in normal animals a proportionate increase occurs in the value of each of the component lipids. During certain experiments recently performed in this laboratory, a group of some two dozen healthy, normal, white leghorn cockerels became available, and it appeared desirable to make a further study of the interrelation of plasma lipids in these birds belonging, as they do, to a class of vertebrates different from that usually experimented upon in studies of lipid metabolism. A differential lipid analysis of the blood plasma of cockerels has not been made with oxidative micromethods. The few studies of blood lipids in birds that have been done have been concerned chiefly with the effect of egg-laying (e.g., 9, 11) and in none of these has a complete analysis been performed.

It was also felt that an analysis of the plasma lipids of birds would provide information of value in connection with another aspect of lipid metabolism, namely, the relation of plasma lipids to diet. The lipid content of plasma is in general somewhat, and often markedly, lower in herbivorous animals such as rabbits (4), guinea pigs (6), goats (1), cows (8), and steers (10) than in omnivorous animals such as man (3) and dogs (7). Bloor (2) has found that the blood lipid levels of rabbits may be altered by changes in diet to a greater extent than those of dogs.

Methods

The cockerels used were obtained from a poultry farm in the spring of the year, when they were one pound in weight. They were housed in a large

¹ Manuscript received August 30, 1939.

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room, with complete liberty of movement, and were fed on Purina Chick Growing Mash and whole wheat grain with crushed oyster shell and water ad libitum. They were killed in the early autumn, and weighed between 1200 and 1600 gm. each. At this time they appeared to be in excellent condition, crowded lustily, and had well developed combs. An autopsy was performed immediately after drawing blood for lipid analysis, and there were no pathological findings. Potassium oxalate was used as an anticoagulant for the blood, which was collected from the neck vessels. The blood was immediately centrifuged and the plasma extracted and analysed by oxidative micro-methods (5).

It is an accepted and unfortunate fact that "blood lipid" values vary according to the technique employed, especially colorimetric estimations of cholesterol versus the oxidative method. A discussion of some factors that may affect lipid estimations has recently been made (5). Not only is there a variation from technique to technique, but occasionally two or more analysts may fail to obtain the same results with the same technique. After sufficient experience however, a qualified analyst may repeat his own results within a coefficient of variation of less than 5%. Comparative studies of blood lipids by the same analyst with the same technique yield data which do not require the necessity of estimating the effect of differences in technique.

Results

A statistical summary of the results is given in Table I. Most of the values were notably similar to those in normal human blood, analysed by these same methods, and as notably different from the lipid values of rabbit and guinea

TABLE I

THE LIPID COMPOSITION OF BLOOD PLASMA IN 22 YOUNG WHITE LEGHORN COCKERELS

(Results are expressed in mg. per 100 ml.)

Value	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
Minimum	368	115	248	65	31	19	90
Maximum	704	374	503	154	106	54	240
Mean	520	225	361	100	66	34	155
Standard deviation	85	77	74	23	19	9	34
Coefficient of variation	16	34	20	23	29	26	22

pig blood. The various cholesterol fractions and phospholipid were somewhat lower in value than those in man and the neutral fat somewhat higher. All of the values were considerably higher than previously found by the author in the blood plasma of rabbits and guinea pigs (4, 6). We have to the present determined lipid values in the blood plasma of four different species, and the

various means of these analyses have been collected in Table II. It is hoped that an opportunity may later be provided to extend this comparative study.

TABLE II

A COMPARISON OF THE MEAN VALUES FOR PLASMA LIPIDS IN HUMAN, COCKEREL, RABBIT, AND GUINEA PIG BLOOD BY THE AUTHORS' OXIDATIVE MICROMETHOD

(Results are expressed in mg. per 100 ml.)

Species	Total lipid	Composition of total lipid					
		Neutral fat	Total fatty acids	Cholesterol			Phospho-lipid
				Total	Ester	Free	
Human (3)	530	142	316	152	106	46	165
Cockerel	520	225	361	100	66	34	155
Rabbit (4)	243	105	169	45	23	22	78
Guinea pig (6)	169	73	116	32	21	11	51

In order to show the relation of values of the different lipid fractions one to the other, the results for cockerel plasma were divided into groups of increasing lipid values. Moving averages within these groups were then calculated by averaging the values in groups with total lipid values between

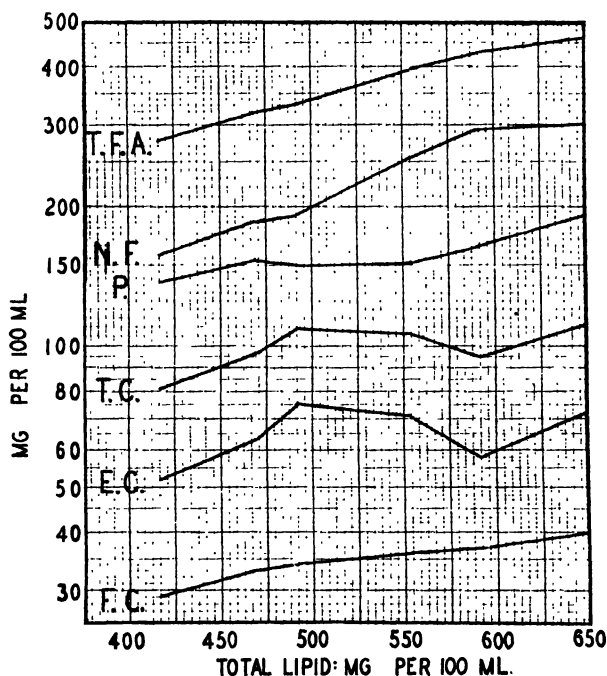


FIG. 1. Arithlog plotting of changes in the moving averages of values for lipids in the blood plasma of healthy young cockerels with changes in the moving averages for total lipid. T.F.A.—total fatty acids; N.F.—neutral fat; P.—phospholipid; T.C.—total cholesterol; E.C.—ester cholesterol; F.C.—free cholesterol.

350 and 450 mg. per 100 ml., between 400 and 500 mg., between 450 and 550 mg., and so on. The resulting moving means were then plotted on arithlog paper and this chart is given in Fig. 1. It may be seen again that in general when there is more of one lipid present in plasma there is correspondingly more of another. As in rabbit plasma (4), the chief fluctuations occurred in the values for ester cholesterol, which erratically increased with increase in total lipid. It should be emphasized again that these interrelations have been demonstrated in *normal* animals. In conditions accompanied by a lipopenia or lipemia, such correlation may not—and in instances where it has been studied does not (3)—exist.

The results indicate further that plasma lipids in normal herbivorous animals need not necessarily be lower than those in omnivorous animals.

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THE CARBOHYDRATE METABOLISM OF DEVELOPING SALMON EGGS¹

BY F. R. HAYES² AND ANDREW HOLLETT³

Abstract

The glycogen and glucose contents of salmon eggs, detached embryos, yolk sacs, and livers have been estimated periodically from fertilization to the end of yolk absorption. Glycogen is absent at the beginning, appears midway between fertilization and hatching, and later increases steadily in concentration, reaching a maximum of 0.53 mg. per 130 mg. egg. Glycogen storage by the liver begins suddenly some time after hatching, before which the muscles serve as a storage place. No glycogen ever appears in the yolk. The initial quantity of glucose is 0.064 mg. per egg. The concentration increases with development, is interrupted at hatching, and then continues to rise, reaching a maximum value of 0.29 mg. per egg. The glucose is distributed throughout all parts of the system, and its fluctuations parallel those of the water content, leading to the suggestion that the glucose concentration per unit of water is constant throughout development.

This investigation was carried out to determine the carbohydrate content of salmon eggs and larvae up to the end of the yolk-sac stage, in order to obtain information regarding the metabolism of carbohydrate and its place of storage. The free sugar content and the glycogen content were determined of: (i) the whole egg or larva (embryo plus yolk); (ii) the embryo only; (iii) the yolk only; (iv) the liver. Where the word "embryo" is used in this paper, it means "embryo dissected off the yolk sac".

Carbohydrate in eggs and animal tissues may be present in three forms, namely free glucose, free glycogen, and a carbohydrate complex forming part of a protein molecule. Of these we have estimated only the first two, so that when we speak of "total carbohydrate" we mean "total free carbohydrate". Of the third fraction, attached to protein, it has been generally assumed that it is absent or negligible in the yolk of chick eggs, and was in fact thought to be exclusively a part of the mucins and mucoids, which in their turn are thought to be essentially confined to the albumen. It is now known, however, to be present in all the fractions of albumen (21) and its presence in chick egg yolk has been reported at least once (6). The complex appears to be made up of four molecules of mannose, two molecules of glucosamine and an unidentified compound of nitrogen (17). (Neuberger (17) found that the carbohydrate could not be split off from the protein by any physical means such as recrystallization, ultra-filtration, denaturation with acid, or denaturation with heat, so that the method used by us would not yield any of it.) Nothing is known of the proteins inside a salmon egg. We are of the opinion that any combined carbohydrate present is not in sufficient quantity to account for the observed rise in free carbohydrate (Fig. 1), although we have no proof of this for the

¹ Manuscript received August 24, 1939.

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salmon. In the frog, however, only 10 to 15% of the carbohydrate is in the combined form (4), while in the chick the combined carbohydrate makes up one-third of the total, and nearly all of it must be in the albumen (13, 14). The only salmon egg protein which has been investigated is that of the shell or casing, in which Young and Inman (22) found that glucosamine made up 1.04% of the dry weight. This quantity would be negligible for embryonic use even if it were passed over to the embryo, a point on which there is no evidence.

Methods

Several thousand recently fertilized, and a smaller number of unfertilized, eggs of the Atlantic salmon, *Salmo salar*, were obtained from the Government Fish Hatchery at Bedford, N.S., at the time when the eggs were being fertilized in November of 1937 and 1938. The fertilized eggs were reared in a hatchery at the University at an average temperature of 7° C., and determinations of the glucose and glycogen content of eggs or larvae were made weekly. Carbohydrate analyses of detached yolks, embryos, and livers were also periodically made.

The sum of the carbohydrate present in the detached embryo and yolk was less than that obtained from an analysis of the whole larva. This was to be expected since the detachment of the yolk sac caused the rupture of all blood vessels connecting the embryo with the yolk, which necessarily must have resulted in a slight loss of blood. The error for glycogen was not great, but the glucose loss was sometimes 10 to 20%. However, analyses of whole larvae were usually made at the time that carbohydrate determinations of egg fractions were carried out, and Table I has been constructed from data of both kinds.

It was found that the wet weight of eggs varies from year to year; in 1937 the average weight of recently fertilized eggs was 116 mg.; in 1938 the weight was 149.75 mg. Hayes and Ross (8) encountered a like variation in weights. They therefore invented a standard fertilized egg of 130 mg. wet weight and adjusted their figures on this basis. A similar procedure was followed in this investigation.

In the results to follow, ages have been expressed as the number of days before or after hatching. Introduced by Allen (1), this method of expressing the age of the salmon eggs and larvae decreases the error caused by varying developmental rate from year to year due to temperature variations of the water in the aquarium. The date on which most eggs hatched was taken to be the zero day or central date of hatching.

The carbohydrates present were obtained by Soxhlet extraction with water carried out for 3 to 5 hr. Material to be extracted was first placed in boiling water to destroy any enzymes capable of converting glycogen to glucose (12, p. 185). The number of eggs or larvae used in a test varied from 20 to 40 depending on the carbohydrate content.

Because of the solubility of glycogen in water (7) it was possible to estimate both glycogen and glucose from a single sample. Each extract was divided

into two portions. On one the glucose was estimated directly; in the other the glycogen was converted to glucose by acid hydrolysis before the glucose determination. The glucose value of the second portion, minus that of the first portion, multiplied by 0.927 gave the glycogen concentration (16). The customary factor for conversion of glucose to glycogen is 0.927, although Kerly (9) recommended 0.957, while Kerr (10) found the factor in acid hydrolysis to be 0.938.

Sugar was estimated by the method of Patterson (18), whose procedure was slightly modified by the use of 2 ml. of aqueous extract instead of 0.2 ml. By performing the experiment with standard glucose solutions a series of values was obtained from which a graph similar to that given by Patterson was constructed. The method is basically that of Hagedorn and Jensen, from which it differs only in the manner of determining the amount of reduced ferricyanide.

The Carbohydrate Content of Eggs and Whole Larvae

Determinations were made from the day of fertilization until the yolk sac had completely disappeared, 92 days after hatching. The results are indicated in Fig. 1 and in Table I. Glucose plus glycogen equals total carbohydrate.

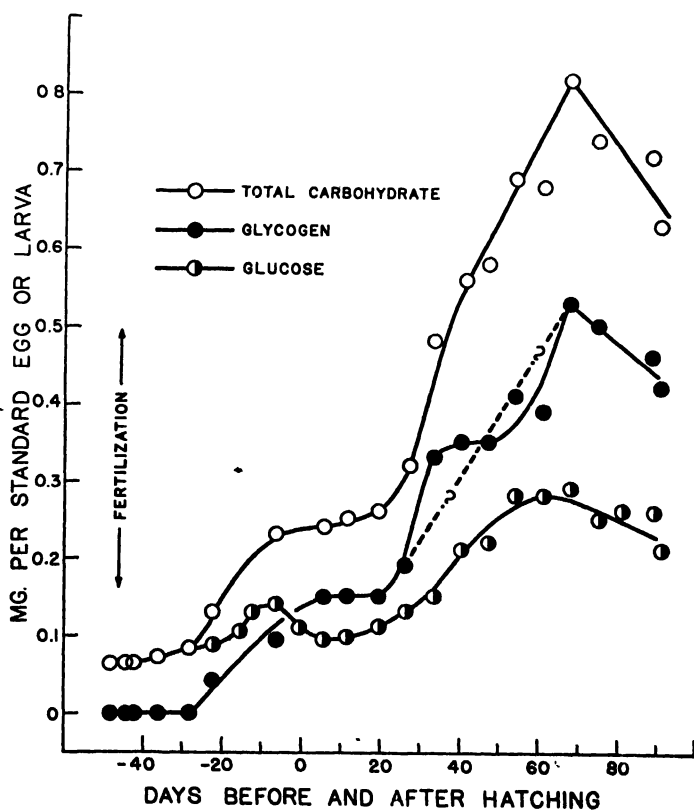


FIG. 1. Distribution of carbohydrate in the whole egg or larva.

TABLE I

RESULTS OF ANALYSES OF SALMON EGGS, LARVAE, AND CONSTITUENT PARTS OF THE SYSTEM
All figures are in milligrams per egg, assuming that an egg weighs 130 mg. in the early stages

1	2	3	4	5	6	7	8
Days before and after hatching	Yolk sac glucose	Embryo glucose	Total glucose	Embryo or total glycogen	Total carbohydrate	Liver glucose	Liver glycogen
Unfert. eggs			0.064	0.000	0.064		
-44			0.065	0.000	0.065		
-42			0.066	0.000	0.066		
-36			0.071	0.000	0.071		
-28			0.083	0.000	0.083		
-22			0.089	0.041	0.13		
-15			0.104				
-12	0.10	0.028	0.13				
- 6			0.14	0.094	0.23		
0	0.085	0.025	0.11				
6	0.062	0.033	0.095	0.15	0.24	0.004	0.01
12			0.099	0.15	0.25		
20	0.051	0.045	0.11	0.15	0.26		
27			0.13	0.19	0.32	0.0027	0.0073
34	0.053	0.084	0.15	0.33	0.48		
41			0.21	0.35	0.56	0.0028	0.04
48	0.07	0.13	0.22	0.35	0.58		
55			0.28	0.41	0.69		
62	0.10	0.14	0.28	0.39	0.68	0.0043	0.10
69			0.29	0.53	0.82		
76	0.083	0.14	0.25	0.50	0.74	0.0062	0.15
82			0.26				
90			0.26	0.46	0.72	0.009	0.22
92			0.21	0.42	0.63		
Yolk sac gone							

The carbohydrate content of eggs two days after fertilization was 0.065 mg. This was all in the form of free sugar; no glycogen was found until three weeks after fertilization. The amount of glucose in unfertilized eggs was estimated to be 0.064 mg. These determinations were made on four batches of eggs varying from 25 to 35 in number. The first two batches contained an average of 0.063 mg. of glucose, while an average of 0.064 mg. was present in the second two. Duplicate analyses were nearly always made of whole eggs. Since all glycogen in advanced stages was in embryos only, therefore the same was assumed to be true in the early stages when the embryo could not be dissected from the yolk sac. This assumption gives the early points in the embryonic glycogen curve as drawn (Fig. 3).

Except during the period of hatching, there is an increase in carbohydrate from the time of fertilization up to about 70 days after hatching when nearly all the yolk has been absorbed. The maximum concentration is 0.82 mg. per larva. There was a continued decrease in carbohydrate after this time.

A close parallel was observed between increase in glucose and in glycogen in the complete larval system, though the maximum concentration of glycogen is reached a few days after that of glucose.

From a week before hatching to a week after it there is a diminution in glucose. There is, however, no decrease in glycogen, although the curve flattens for three weeks after hatching, following which there is an uninterrupted synthesis of glycogen up to about 70 days, after which it declines.

It is notable that Hayes and Ross (8) found a drop in fat content of the embryo at hatching. Moreover Saunderson (19) found that cartilage formation in the salmon head ceased abruptly at the time of hatching. A comparison of the cartilage in the head and the carbohydrate in the larva is made in Fig. 4. It will be seen that the synthesis of carbohydrate ceases abruptly before hatching and is resumed about three weeks later. In cartilage formation also the curve flattens out for nearly three weeks, after which there is a rapid rise until it reaches a maximum. The similarity of the curves lends support to the suggestion that at hatching or birth there is a pause in the building of new structures, and in the synthesis of new materials. We do not mean to suggest by Fig. 4 that carbohydrate and cartilage have a causal relation to one another, but we do suggest that carbohydrate or fat are morphological entities in the embryo, subject to the same developmental laws as cartilage or any other tissue or organ.

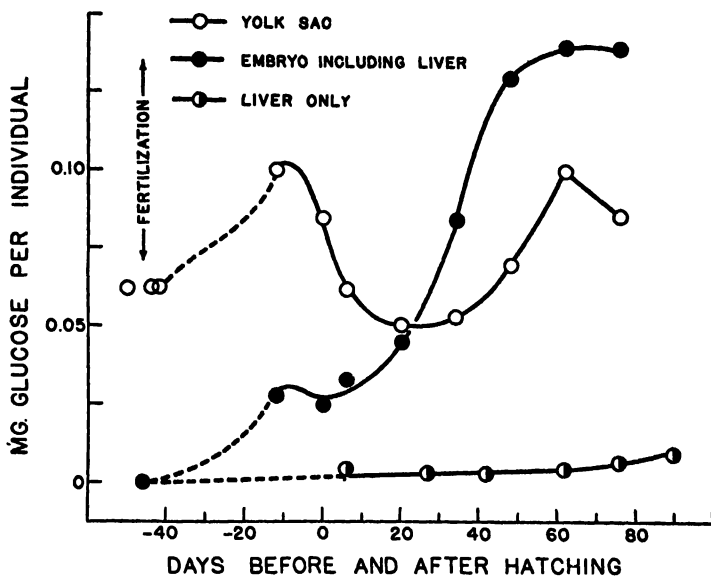


FIG. 2. *Distribution of glucose among the parts of the system.*

From a further examination of Fig. 1 it is evident that the mechanism for the building up of glucose is present from the beginning, since there is an increase in glucose a few days after fertilization. Analyses for glycogen, however, gave negative results until 24 days after fertilization. In duplicate tests a week before this no glycogen was found. This suggests that the mechanism for the synthesis of glycogen is not present in the egg soon after fertilization, but appears somewhat suddenly about three weeks before hatching.

Carbohydrate in Embryos

Determinations of the amount of glucose and glycogen present in the embryos (yolks detached but livers present) were made 12 days before hatching. This was the earliest time at which it was found possible to detach sufficient embryos to make an analysis. After that time the carbohydrate content of embryos was measured about every two weeks. It will be seen from Fig. 2 that glucose rises steadily, sloping off somewhat at hatching, following which it increases fairly rapidly and uniformly until some 60 days after hatching. A similar trend is noted for glycogen (Fig. 1) but the peak is reached 12 days later. It should be noted that "embryonic glycogen" is the same as "total larval glycogen", since all the glycogen is in the embryo.

We have been uncertain what value to give to the apparent flattening of the glycogen points between 30 and 50 days (Fig. 1), so we have drawn the curve both to show the flattening (solid line) and to suppress it (dotted line). In Fig. 3 the "embryo minus liver" curve is derived by subtraction of liver glycogen from the curve in Fig. 1, and in Fig. 3 the two glycogen possibilities are shown between 20 and 70 days.

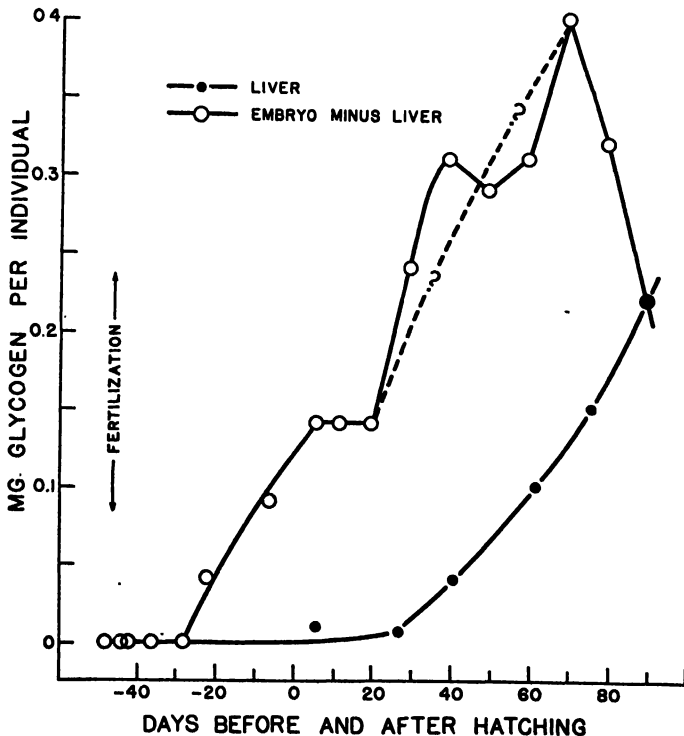


FIG. 3. Distribution of glycogen between the liver and the rest of the embryo. The sum of these two curves appears in Fig. 1 as glycogen in the whole egg. Values for "embryo minus liver" have been obtained by reading from the Fig. 1 glycogen curve, and subtracting the liver curve. In the period from 20 to 70 days, the solid and dotted lines correspond respectively to the same region of the glycogen curve in Fig. 1.

Detached Yolk Sacs

The carbohydrate in the yolk was estimated at the time that analyses were made of the embryos. The values are given in Fig. 2. The glucose in the yolk reaches a peak twice during larval development: about a week before hatching, and at 62 days when ordinarily the larvae would probably be feeding. As already stated, the earliest analyses of detached embryos and yolks were made 12 days before hatching. The curves in Fig. 2 to the left of -12 days have been drawn in dotted lines, their shape being based on the following evidence: at fertilization all the 0.65 mg. of egg glucose must have been in the yolk, since there was no embryo. Similarly, the embryonic glucose at fertilization was zero. Between fertilization and -12 days the sum of the embryonic and yolk curves must give the values in Table I, column 4.

When the last analyses were made the yolk was almost completely absorbed, and the sac could be detached only with great difficulty. At the time of the final analysis of larvae at 92 days the yolk sac had completely disappeared.

No glycogen was detected in the yolk sac except on two occasions, and then in such minute quantities—of the order of 0.01 mg.—that it might well be attributed to error. Therefore, all the glycogen present in the larval system was stored in the embryo.

Livers

Periodic estimations were made of the carbohydrate content of the liver. The results are shown in Figs. 2 and 3. Only minute quantities of reducing sugar were found. An examination of the graph indicates that the liver

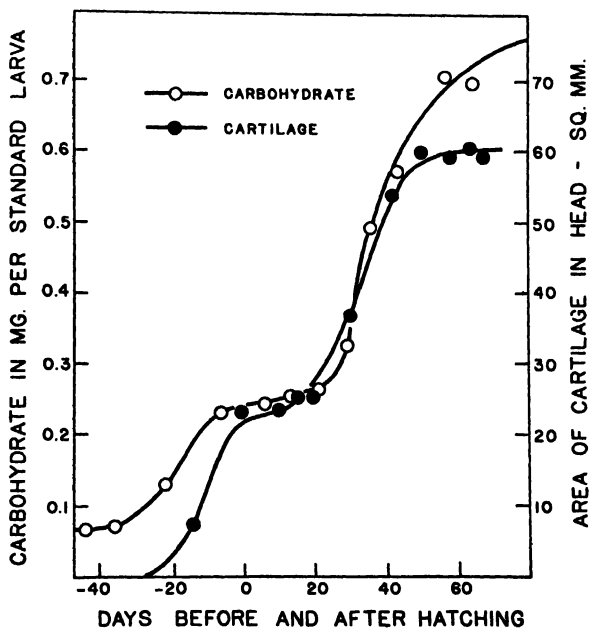


FIG. 4. A comparison of the total carbohydrate in a developing salmon larva with the area of cartilage in the head. Cartilage values are from Saunderson (19).

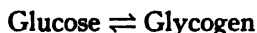
does not act as a storage place for glycogen until some 30 to 40 days after hatching, when this function is rather suddenly assumed. After this time the glycogen content rapidly increased until the last analysis was made 90 days after hatching, when 0.222 mg. were stored there. This rapid rise in glycogen will be discussed later.

Discussion

From Fig. 2 it will be observed that the embryonic and yolk glucose tend to rise and fall together. The simplest explanation of this is found in the suggestion that glucose in an embryo, as elsewhere, is not stored in any specific tissue, but passes freely through all walls and is distributed throughout the larval system as an aqueous solution equally concentrated in all parts. According to this interpretation the ratio of glucose between embryo and yolk at any time serves merely as an index of the relative quantities of water in each. It also follows that at any time, the embryo with 80 to 90% water will contain more glucose per gram wet weight than the yolk with 50 to 60% water.

Fig. 2 shows that the synthesis of glucose from some source (not glycogen) begins at the time of fertilization. It is presumably accomplished by means of enzymes within the embryonic cells and will therefore be proportional in intensity to the number of such cells. This synthesis of glucose never stops during development, although it is quite possible that its rate diminishes for a short time before hatching. Against this continual synthesis we have to set (a) the utilization of glucose as fuel, which undoubtedly goes on all the time although we cannot express it quantitatively, and (b) the removal of glucose as glycogen. The capacity for glycogen formation is not, like that of glucose, present from the beginning, but appears rather suddenly some 28 days before hatching. Histochemical studies showed that the place of storage was chiefly the muscles; those of the caudal and dorsal fins stained markedly with 0.1 *N* iodine solution. Probably the onset of glycogen storage corresponds to the time in embryonic development when muscle tissue becomes differentiated. Glycogen storage by the liver is discussed below, but it may be noted here that during the whole larval period muscle storage is much the more important part, an observation which points to the danger of interpreting embryonic glycogen as a demonstration of liver storage.

In Fig. 1 a rather constant time lag between glucose and glycogen fluctuations will be noticed. Thus glucose begins to diminish some eight days before hatching, and two weeks later the synthesis of glycogen stops. Six days after hatching glucose is synthesized again, and two weeks later the same thing happens to glycogen. At 60 days the glucose curve again turns down, and again 10 days later the glycogen curve follows. This last change happens in spite of the fact that the liver glycogen pursues its course unaltered. The suggestion comes very readily that we are dealing in the muscle cells (though not in the liver) with a reaction



which may proceed in either direction depending on the relative concentrations of the two substances. The embryo is therefore taking any of the new glucose that is left over after metabolic needs are satisfied and converting it into muscle glycogen. In time of need this muscle glycogen is drawn on as a reserve. The final drops in all three curves after about 70 days are to be interpreted as the onset of starvation, for at this time the yolk is almost used up. By the end of our observations at 92 days there was no yolk sac visible at all.

It is interesting to find that Shaw (20) noted the disappearance of glycogen from the livers of chicks on the day of hatching, although glycogen was present in the liver the day before and two days after hatching.

One of the most striking things in these analyses is the sudden appearance of glycogen in the liver some 40 days after hatching, and its rapid rise after this time. Fig. 3 represents the glycogen in the liver and in the embryo minus liver. The curve for the embryo without liver rises steadily until the 68th day, and then declines sharply. This corresponds to the time when the storage of glycogen by the liver is taking place most rapidly. The yolk has not yet been completely absorbed, and there is still an increase in total embryonic glycogen.

The sudden glycogenic function of the liver has been noted by many investigators, and appears to be almost universal among the vertebrates. Lochhead and Cramer (11) found that though definite amounts of glycogen were present in foetal livers of rabbits, the percentage was very low up to the 25th day. This represented the time when the liver assumed its adult glycogenic function. They noted that this coincided with the beginning of the decrease in placental glycogen.

In studies on various embryos, Aron (2) showed that in the pig the liver functioned as a storage place for glycogen near the end of gestation. In the sheep, glycogen is found in appreciable quantities at the end of the fourth month of pregnancy, and in the guinea pig the glycogenic function is assumed more slowly but definitely in the last four days of gestation. He discussed the appearance of the islets of Langerhans out of the islets of Laguesse, and found a striking coincidence between the appearance of the islets of Langerhans in the pancreas and the deposition of large quantities of glycogen in the liver. Moreover, he endeavoured to show that the islets of Langerhans produce insulin, but those of Laguesse do not. From further investigations by histochemical methods Aron (3) found no glycogen in the liver before the appearance of the hind limb buds, and that removal of the pancreas in early stages prevented the appearance of glycogen in the liver. As a result of his research, Aron concluded that the beginning of this function of the liver varies from species to species but manifests itself at a fixed time in ontogeny. The present work supports his theory.

There is, of course, no pancreas in teleosts, but in the adults there are well-defined masses representing the islets of Langerhans. Whether the appearance of glycogen in the liver of the salmon is related to islet tissue must remain for

the present a matter of speculation, since no reference has been found to the time of its appearance.

There is a synthesis of carbohydrate from fertilization, uninterrupted except at the time of hatching, until the beginning of the final starvation period. We have examined the literature but can find no other case in which the embryo ends up with more carbohydrate than the egg had at the beginning. This unique property of the salmon egg is no doubt shared by other teleosts. We do not know what the source of this new carbohydrate is. Certainly the great period of carbohydrate synthesis after hatching corresponds to a sharp drop in fat (8), although, since the carbohydrate gain amounts to only one-tenth the fat loss, the two cannot be said to be quantitatively related. For the three weeks before hatching there is a synthesis of both fat and carbohydrate, although the absolute quantity of carbohydrate involved is quite small, of the order of 0.1 mg. per egg. This gain might be accounted for by the combined carbohydrate in protein molecules or by the protein itself.

A synthesis of glycogen (as distinct from total carbohydrate) is found in the chick as well as in the salmon (13). In the salmon the glycogen is all in the embryo. In the chick, however, Needham divided the system into "embryo" and "remainder of the egg". The latter part included not only the yolk and albumen, but the allantois, amnion, and the coelom with its two walls each composed of two germ layers. It is not surprising therefore that the newly formed glycogen was found in both parts. Needham does not suggest, nor do we see any evidence for, the storage of glycogen in the yolk or albumen. Its storage as in the salmon is evidently cellular, the extra embryonic portion being laid down in the blastoderm. In our method of separating the yolk sac from the salmon embryo, the division was made in the coelom, so that the somatic mesoderm and ectoderm were included with the embryo, the splanchnic mesoderm and endoderm with the yolk sac. The quantity of cellular material which went with the yolk sac was a very small proportion of the whole embryo. The glycogenic function of the extra embryonic membranes of the chick is probably something new which has arisen with the membranes themselves.

The view has been expressed that glucose traverses cell walls and is found everywhere in the salmon system, including the yolk. Glycogen on the other hand is evidently confined to cells. If the enzymes necessary to synthesize glycogen act only in cells, it is, to say the least, probable that the enzymes which promote the production of glucose from glycogen also act in cells. They may very well be the same enzymes. With this distinction between glucose and glycogen in mind, we may examine Table II, which shows the stores of carbohydrate with which different eggs start out. So far as investigated, eggs of birds, reptiles, and fishes, have their initial store as glucose, with little or no glycogen. Amphibia and invertebrates on the contrary have stores of glycogen with little or no glucose. The only exception is the trout egg, in which Fauré-Fremiet and Garrault report 0.34% glycogen. About the same time as the trout work, however, Fauré-Fremiet and his collaborators were

TABLE II

THE CONCENTRATIONS OF GLUCOSE AND GLYCOGEN IN VARIOUS EGGS AT THE TIME OF FERTILIZATION

All figures are in per cent wet weight

Animal	Glucose	Glycogen	Author
Chick, whole egg	0.20	0.003	Various authors
Chick white	0.50	0.003	Sakuragi
Chick yolk	0.33	0.002	Sakuragi
Tortoise	0.14		Diamare
Frog		2.5	Various authors
Herring	0.50		Steudel and Osato
King salmon	0.096		Greene
Atlantic salmon	0.050	0.000	This paper
Trout		0.34	Fauré-Fremiet and Garrault
Starfish		0.020	Dalcq
Sea urchin (<i>Echinus</i>)		1.3	Moore, Whitley, and Adams
Sea urchin (<i>Strongylocentrotus</i>)	0.08	1.28	Ephrussi and Rapkine (5)
Silk worm		1.5	Various authors
Bee		2.5	Straus
Sabellaria (Polychaet worm)		1.3	Fauré-Fremiet

NOTE.—Chick figures are from Needham (15, Vol. 1, p. 278), other figures are from Needham (15, Vol. 1, p. 356), except *Strongylocentrotus* (for which see (5)) and our salmon figures.

reporting glycogen concentrations in frog eggs much greater than those found by others—sometimes four or five times as high as the average of other workers. For this reason we are inclined, pending confirmation, to disregard the trout figure.

If we look for an underlying morphological explanation for Table II, we find that the "glycogen" eggs are those in which the whole egg divides into cells that participate in development. In the frog, for instance, though the cells in early development differ markedly as to size, each cell will contain a store of glycogen. One does not think of the embryo as taking material from the yolk, but rather of the yolk cells continuing to divide and changing gradually into differentiated embryonic cells. The stores are therefore divided from the outset.* There is a time rather early in development when most of the activity is in small cells with limited stores, and conversely, most of the stores are in the relatively inactive yolk cells. It is during this time that the active cells, with the glycogen store (as we think) locked away from them, use up most of the available combined carbohydrate (4). Later the problem solves itself by cell division, which breaks up the storage cells and distributes them; and by the acquisition with continued differentiation, of glycogen-splitting enzymes in the former yolk cells. The same argument applies with even fewer provisos to the eggs of echinoderms, while in the insects the nucleus continues to divide without cell walls until the several hundred nuclei migrate to the surface and cell walls are formed. All the egg material

* The division of stores is clearly illustrated by unpublished microphotographs of *Amblystoma* larvae which Dr. Herbert Coar has kindly shown us. Here the muscle cells, after the differentiation of fibres and cross striations, still contain numerous yolk platelets.

therefore participates in early development. The polychaetes have total spiral cleavage, so that again there is nothing corresponding to a yolk sac.

Turning to the "glucose" eggs of birds, reptiles and teleosts, it is found that the embryonic stores are locked away in the large, non-cellular yolk sac. As the small embryo uses up glucose it is replaced by simple diffusion from the yolk, a mechanism that would not serve if glycogen made up the carbohydrate store. The glucose taken by the embryo is constantly replaced and augmented, although we do not know where or how. Now if we were to take a salmon egg, which is about half water at the beginning, and by the addition of water to make it two and one-half times as heavy as it was before, it is obvious that there would be four times as much water as before, and that if the glucose per egg remained the same, the glucose tension would drop to one-quarter its former value. If at the same time as the water, we added enough glucose to make a fourfold increase per egg, then the glucose tension would remain unchanged. In a salmon larva at the end of the yolk sac period there actually is four times as much water, and as Table I, column 4 shows, four times as much glucose, as well. We are inclined to conclude that the glucose tension throughout development remains the same, and that, as already mentioned, when glucose tends to exceed the fixed level glycogen is formed, and vice versa. It will be possible to formulate this theory quantitatively when we have a good set of wet and dry weights. At first sight the chick appears to develop in the opposite manner, since there is a diminution in glucose per egg during development, but it must be remembered that the chick gets drier, not wetter as it develops, and moreover the part played by the amniotic fluid with respect to glucose is not known. It is quite possible that the glucose tension in terms of embryonic chick water is constant throughout development.

In conclusion mention may be made of the rate at which new carbohydrate is formed in the salmon. We cannot do this quantitatively, but an inspection of Fig. 1 shows that the curve for total carbohydrate is steepest in the periods -28 to -6 and 24 to 45 days. These probably correspond to the periods of maximum synthesis per unit wet or dry weight of embryo. The first of these fits very well with the table of anticipated energy sources previously published (8, p. 372). The second maximum, however, is something new and we do not at the moment see that it has any correspondence in chick embryology.

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PARASITES OF FRESHWATER FISH

II. PARASITISM OF SPECKLED AND LAKE TROUT AND THE FISH FOUND ASSOCIATED WITH THEM IN LAKE COMMANDANT, QUE.¹

BY L. L. LYSTER²

Abstract

Parasitism of two game fish, *Salvelinus fontinalis* and *Cristivomer naymaycush* and of two other fish, *Perca flavescens* and *Catostomus commersonii* is discussed. *Glaridacris intermedius* sp. nov. (Cestoda), *Rhabdochona laurentiana* sp. nov. (Nematoda) and *Raphidascaris alius* sp. nov. (Nematoda) are described.

In presenting further information on parasitism in freshwater fish in the Province of Quebec it is hoped that a contribution will be made not only to distribution records, but also to the interrelation of parasitic forms. The first paper of this series (13) dealt with general trematode parasites. Collecting was done over an extended period and in a considerable area, and although several trematodes were found in two or more hosts, it was impossible to judge what part any fish might play in maintaining an infection in another. The present paper, on the other hand, is based on material collected in limited and known waters and within a short period of time. This material was collected during the months of September and October, 1938, in Cameron's Bay, a southerly projection of Lake Commandant.

Four hosts are considered, *Salvelinus fontinalis*, *Perca flavescens*, *Catostomus commersonii*, and *Cristivomer naymaycush*. Of these, the first three, speckled trout, yellow perch, and common sucker, were invariably found together. The fourth, lake trout, was never found in association with the other fish, but was taken in the deeper parts of the same waters; it was included in the survey in the hope that its parasite records might indicate whether identical parasitism was more apt to occur among totally unrelated fish of similar habitat, or related fish living under different conditions.

TREMATODES

Two species of *Crepidostomum* were collected, one from perch and one from speckled trout. In addition a trematode reported as a new species from perch in the first paper of this series was found in small numbers in the perch and in considerable quantity in the trout. Both suckers and lake trout were free of trematodes. Metacercariae of a member of the genus *Apophallus* were present in large numbers in the skin of speckled trout, and metacercariae of a strigeid were noticed in small numbers in three of the perch. These immature forms will be discussed later.

¹ Manuscript received July 22, 1939.

Contribution from the Institute of Parasitology, Macdonald College, Que., with financial assistance from the National Research Council, awarded through the National Committee on Fish Culture.

² Graduate assistant.

Crepidostomum isostomum Hopkins, 1931Host: *Perca flavescens*

Material: three specimens

These hexapapillate forms have features that suggest identification as either *Crepidostomum isostomum* Hopkins 1931, or *C. canadense* Hopkins, 1931. Measurements of major structures are very similar to those of *C. isostomum*, but are consistently greater, and uniformly twice those for *C. canadense*, except that of width. The ratio, length of pharynx to diameter of oral sucker, the shape of the ovary and its position in relation to the ventral sucker are different from *C. isostomum* but similar to *C. canadense*. The post-testicular condition of the vitellaria is unlike that described for *C. canadense* but like *C. isostomum*. With these exceptions, all considered variable, the present material could be assigned to either of the species. *C. isostomum* and *C. canadense* are thought by their author to be closely related (8), and since this material seems to possess characters of both species, *C. canadense* has been listed as a synonym of *C. isostomum*.

Three of these trematodes were recovered from a single yellow perch from the lake. They were long, narrow forms 2.5 by 0.575 mm., widest and deepest in the ovarian region, tapering to a point terminally and narrowing to a neck posterior to the prominent hexapapillate oral sucker. Oral sucker and acetabulum uniformly round, 0.25 mm. in diameter. Reduced pre-pharynx. Pharynx slightly longer than broad, 0.074 by 0.098 mm. Oesophagus long, opening in caeca dorsal to acetabulum. Caeca terminating behind testes. Cirrus sac a short wide structure curving slightly dorsally but only slightly exceeding or not quite reaching anterior margin of acetabulum; 0.25 mm. in greatest measurement, opening into common genital pore anterior to intestinal bifurcation. Ovary lateral, globular, removed posteriorly from acetabulum by about its own diameter, 0.24 mm. Globular seminal vesicle contiguous with posterior margin of acetabulum to dorsal of ovary, slightly larger than that organ. Testes irregularly shaped, margin entire, medial, slightly oblique and posterior to ovary and receptacle, about 0.28 mm. in diameter; considerably removed from posterior end of body, close together but not contiguous. Vitellaria extend from cirrus sac to posterior end of body in lateral medial area, few follicles dorsally and meeting posteriorly but not overlapping behind testes. Uterus with few small eggs, convoluted in region of ovary and looping to anterior margin of anterior testis.

Crepidostomum cooperi Hopkins, 1931Host: *Salvelinus fontinalis*

Material: 50 specimens

The anterior extent of the vitellaria, the relative size of the pharynx and suckers, and the posterior position of the crural furcation are confusing details in these forms, as well as the comparatively large size of the eggs. The body size, the vitellaria, and general organization are characteristic of *C. cooperi*,

particularly the variant described as *C. fausti* by Hunninen and Hunter (10). The absolute measurements of the sucker and egg are suggestive of *C. farionis*, but, in proportion to body size, they are larger. The fact that the body is recurved and somewhat contracted (but not shrunken) anterior to the acetabulum, would perhaps explain the rather posterior position of the crural furcation and the more anterior genital opening than is typical, as well as the extensive vitellaria in this region. This condition and the extreme variability of the species will not allow for a diagnostic importance for these atypical details, and the form has, therefore, been listed as *C. cooperi* Hopkins, 1931.

They are compact, broad, thick forms, 0.70 to 1.49 by 0.25 to 0.56 mm., body widest at the level of the oral sucker or at the acetabulum, or sides of body almost parallel and the ends bluntly rounded, thickest at the posterior margin of the acetabulum, and usually ventrally curved. Oral sucker subterminal 0.11 to 0.21 by 0.14 to 0.25 mm. with six non-prominent papillae rarely extending past limits of the body. Pharynx globular, about 0.08 to 0.015 mm. in diameter, dorsal and posterior to oral sucker, prepharynx, and pharyngeal vestibule. Oesophagus short, bifurcation at anterior margin or slightly in front of it. Crura extending well posterior to testes. Genital pore ventral to intestinal furcation. Cirrus sac long, slender, widest posteriorly passing dorsally to acetabulum to its posterior margin or slightly beyond, never filled by seminal vesicle. Acetabulum larger than oral sucker, 0.14 to 0.28 by 0.17 to 0.35 mm., greatest dimension laterally, medial, well anterior, and often almost in contact with oral sucker. Ovary globular, slightly lateral, dorsal of posterior margin of acetabulum, or slightly more posterior, 0.7 to 0.14 mm. Seminal receptacle varying in shape and size, and medial or sub-medial, not far behind ovary. Testes two, tandem, and globular or slightly wider than long, margins entire, 0.08 to 0.17 by 0.15 by 0.18 mm. removed by at least one diameter, and usually more, from terminal area of body. Uterus with many large intra-uterine eggs about 56 by 84 μ extends as far as midpart of posterior testis. Vitellaria of large closely packed follicles filling all available space from anterior to posterior limits of body but somewhat limited dorsally. Excretory bladder obscured by these structures.

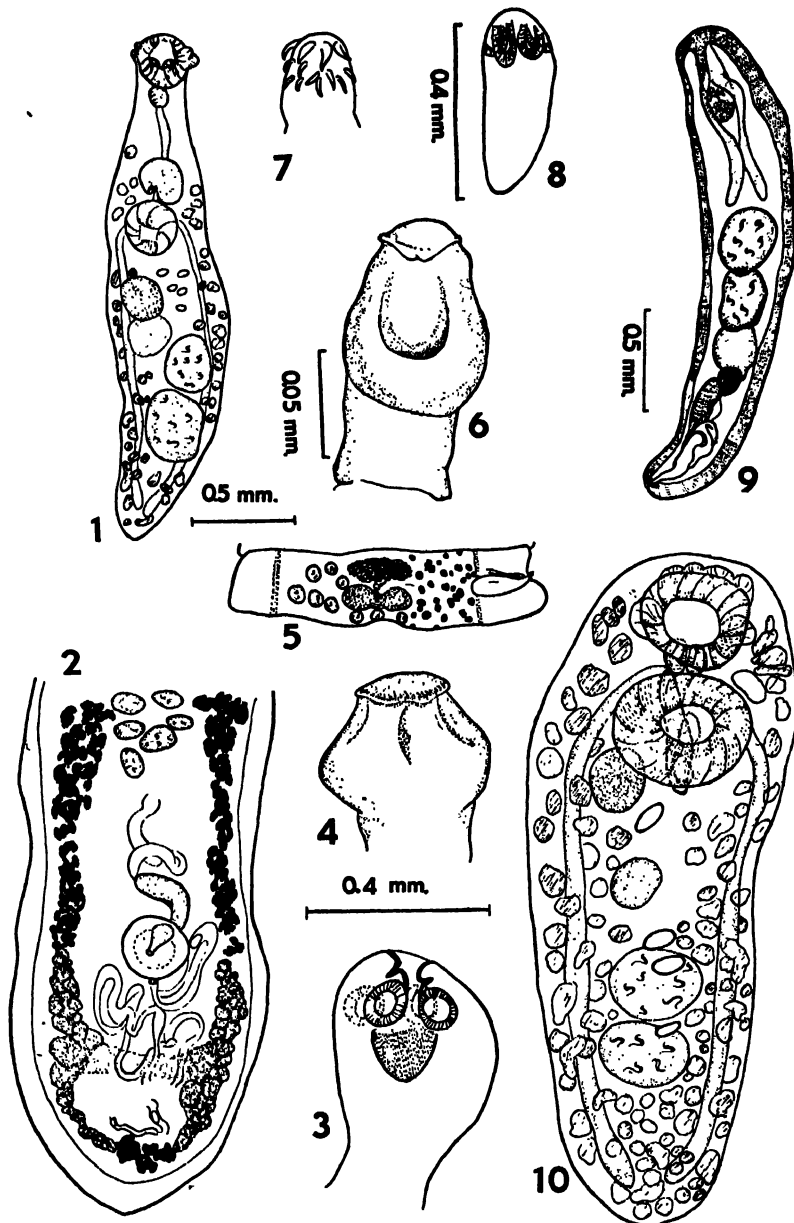
This species has already been reported from this host in Quebec by Richardson (15) under the synonymous name, *C. fausti* Hunninen and Hunter, 1933.

Ptychogonimus fontanus Lyster, 1939

Hosts: *Perca flavescens*, *Salvelinus fontinalis*

Material: 25 specimens

This species was established in the first paper of this series from limited material collected from a perch (13). Many specimens were since collected from *Salvelinus fontinalis*, in the stomach and upper digestive tract. Little can be added to the original description of the species. In the present material it is noted that an oesophageal structure is present as a short, weak, saccate body, opening dorsally from the pharynx, and leading laterally and



FIGS. 1-10. Trematodes, Cestodes, and Acanthocephala. 1. *Crepidostomum isostomum* from *Perca flavescens*. 2. *Glaridacris intermedius* sp. nov. posterior end, from *Catostomus commersonii*. 3. *Ichthyotaenid* from *S. fontinalis*. 4. *Glaridacris intermedius* sp. nov. anterior end, from *C. commersonii*. 5. *Eubothrium salvelini* mature proglottid from *S. fontinalis*. 6. *Eubothrium salvelini* scolex from *S. fontinalis*. 7. *Neoechinorhynchus cylindratu*s—proboscis from *S. fontinalis*. 8. *Ichthyotaenid* from *Cristivomer naymaycush*. 9. *Neoechinorhynchus cylindratu*s, entire worm from *S. fontinalis*. 10. *Crepidostomum cooperi* from *S. fontinalis*.

anteriorly into the crura, a condition similar to that noticed in the related genus *Hirudinella* (4). The crura are naturally convoluted and much longer than the body cavity. This is uniform in all specimens and not due to contractions as previously suggested.

CESTODES

Polyzootic cestodes were limited to the trout. Adults of a single species and immature forms of this genus and another were found in *Salvelinus*. Similar immature cestodes were taken from the lake trout. Monozootic cestodes of a hitherto undescribed species were recovered from the common sucker. Perch carried no cestode infections. By far the heaviest parasitism noted was that of the polyzootic cestodes in speckled trout. All specimens of this fish carried some of the parasites and most had large numbers.

Eubothrium salvelini (Schränk, 1790)

Host: *Salvelinus fontinalis*

Material: 30 specimens

Presence of non-armed and non-specialized pseudobothridia, terminal disc, lateral genital opening, and saccate uterus show they belong to the genus *Eubothrium*. The shape of the scolex, length of the cirrus sac, and organization of vitellaria refer them to the species *E. salvelini* (Schränk, 1790), following the system of classification of Joyeux and Baer (12).

Scolex longer than wide, widest behind base of prominent pseudobothridia, and bearing bilobed terminal disc. Proglottids wider than long, about 1.68 by 0.7 mm. or greater, and considerably greater when compressed, always wider than scolex and often secondarily divided by transverse fissures. Genital opening lateral, alternating only rarely. Ovary bilobed, posterior central uterus saccate, anterior, central. Testes numerous, more or less spherical, about 0.07 mm. in diameter, occupying lateral intermuscular field and contiguous in neighbouring segments. Cirrus sac about 0.07 mm. at widest point in region of longitudinal nerve, about 0.6 mm. long. Cirrus broad, leaf-like when extruded, with minute spines. Vitellarian follicles small, scattered in central lateral fields. Vagina opening posterior but close to male genital openings.

Glaridacris intermedius sp. nov.

Host: *Catostomus commersonii*

Material: 10 adults

Four species have been listed by Hunter (11) in this genus of monozootic cestodes. Of these, two species, *G. hexacotyle* Linton, 1887 and *G. catostomi* Cooper, 1920, do not possess a terminal disc, while two species, *G. confusus* Hunter, 1929 and *G. laruei* Lamont, 1921, like the present form, have this structure in common, as well as non-operculate eggs and the absence of a genital atrium. Like *G. confusus*, this form has a granular ovary, not distinctly separate from the vitellaria, similar body shape and testes in two

lateral rows. Like *G. laruei* it has 70 testes and the vitellaria tend to enclose them. Measurements, egg size, etc., are much alike in *G. confusus*, *G. laruei* and the present form.

The significance of these similarities and differences is obscure at this time, and may indicate synonymities. The present form is apparently closely related to *G. confusus* and *G. laruei*, but cannot be referred to either. It must, therefore, be assigned to a new species which has been designated *G. intermedius* sp. nov.

The adults range in size from 3.5 to 9.0 mm. in length. They are widest at the level of the cirrus sac, 0.5 to 0.84 mm., and taper terminally to a rounded point, anteriorly to the region of the origin of the vitellaria, 0.4 to 0.55 mm. wide, which follows the narrower neck. The scolex is well differentiated and short, rounded at the junction with the neck, and squared anteriorly; it has three pairs of loculi and a terminal disc. All organs are entirely medullary. Vitellaria originate about 0.35 to 0.84 mm. behind the base of the scolex, extending as far as the ovary and with post-ovarian follicles. The testes are about 70 in number, arranged in two more or less parallel rows from a point slightly behind the origin of the vitellaria to about 0.30 mm. anterior to the cirrus sac; they are irregularly oval in shape, 0.07 by 0.14 to 0.07 by 0.084 mm. greatest diameter transversely. The vitellaria occur chiefly in the lateral areas, but scattered follicles are dorsal to the testes. The vas deferens is highly convoluted and enters a short, curved seminal vesicle dorsal and anterior to the cirrus sac. Cirrus sac small, circular, 0.084 to 0.085 mm. in diameter. Common genital atrium absent. H-shaped ovary varies in size, but wings usually about 0.5 to 0.7 mm. long; lateral branches relatively narrow, medial branch also limited. This organ is granular, finely lobate, similar in appearance to and in contact with lateral and terminal vitellaria. Uterus long, convolute, reaching to posterior extremity of body, and anteriorly to the cirrus sac; vagina medial, ventral. Seminal vesicle not obvious. Female system opens close to posterior edge of cirrus sac. Eggs non-operculate, about 42 by 28 μ .

Larval Cestodes

Larval cestodes of two recognizable types were taken from both species of trout. Plerocercoid forms of *Eubothrium* were found in *Cristivomer naymaycush*. These consisted of a scolex and a short undifferentiated body. It is not possible to suggest the species to which these might belong, or to offer any details of their organization. The rather short scolex and deep pseudobothridia might be associated with *E. salvelini*. In other larval forms from this host four muscular suckers were present. These specimens seemed of two types. One consisted of a simple, saccate structure with four incomplete suckers alone distinguishable. In the other a fifth glandular structure was present; this type was also found in the speckled trout. These probably are referable to the family Ichthyotaeniidae Ariola, 1899 (Syn. Proteocephalidae Larue, 1911) as discussed by Joyeux and Baer (12).

ACANTHOCEPHALA

Acanthocephalids were present only in speckled trout and suckers. Light infections in each were caused by the same species.

Neoechinorhynchus cylindratus Van Cleave, 1913

Hosts: *Salvelinus fontinalis*

Catostomus commersonii

Material: mature males and females were found in considerable numbers in the trout; one of each sex was taken from the sucker.

They were short, straight or slightly curved. Males were 3.0 to 4.9 by 0.6 to 0.82 mm., females about 5.1 by 0.82 mm. (longitudinal measurements exclusive of proboscis), broadest just posterior to the lemnisci. Proboscis longer than broad, about 0.53 by 0.12 mm., armed with three rows of six recurved hooks each. The anterior hooks were 0.4 to 0.45 mm. long, those of the two posterior rows were 0.21 to 0.03 mm. long. The hooks alternate in adjacent rows and are without collars or ornaments.

Embryos numerous and large, 0.07 by 0.10 to 0.12 mm. Lemnisci reach posteriorly almost to testis or almost half of body length. Brain located at base of proboscis receptacle. Testes closely applied, tandem, shortened cylinders, equal in size, 0.34 by 0.37 mm. Cement gland, a single body close behind testes, opening into cement reservoir. Small copulatory bursa present in males though often folded and inconspicuous. Genital openings terminal in both sexes.

NEMATODES

Nematodes are represented in the collection by two species from the speckled trout and one from the sucker. Material from the latter host was adequate, but that from the trout included few specimens and those of a single sex. Identification is, therefore, only as accurate as is possible with limited material.

Lake trout and perch were nematode-free.

Cystidicoides harwoodi (Chandler, 1931)

Host: *Salvelinus fontinalis*

Material: one female; two immature males (uncertain)

This species was described as *Cystidicola harwoodi* from speckled trout by Chandler (2) and as *Metabronema canadense* by Skinner (17) from the same host. The latter author later placed it with *C. fischeri* in her new genus *Cystidicoides*, based on the nature of the post-cloacal papillae (18). This has been accepted by Van Cleave and Mueller (21), who reported it from *Salmo fario*.

The following description is based on the single female specimen.

Length 9.0 mm., greatest width 0.3 mm. Lips two, small and inconspicuous with slight chitination at anterior corners, each bearing two papillae. Mouth simple, leading directly into tubular vestibule. Oesophagus in two distinct divisions; first part convoluted, occupying about 0.8 mm., second part 2.6 mm. long, emptying into simple intestine through a terminal sphincter. Divergent ovaries originate 0.15 mm. behind the oesophagus and just anterior to the anus; they are very convoluted. Bicornate uterus enters vagina just posterior to vulva, posterior uterine branch passes forward past this position to join vagina more or less parallel with anterior branch. At the level of the vulva the body diameter is sharply reduced by 0.112 mm. and on this projection the vulva opening is placed. Intra-uterine eggs 27 by 57 μ ; no terminal filaments noted. End of body bluntly rounded 0.116 mm. posterior to anus.

Two immature male forms slightly under 7.0 mm. in length and 0.14 mm. wide at the base of the oesophagus may belong to this species. The relationship is, however, not definite. The mouth and alimentary canal are essentially as in the female. The genital organs are poorly differentiated but a testis originates a short distance behind the oesophagus. The two spicules are dissimilar. The right member is short and wide, about 0.16 mm. long, pointed terminally but widening quickly to make the sides almost parallel; it is concave during the first three-quarters of its length and constricted just before the base. The left spicule is about 0.47 mm. long and narrow, with a widened base and a narrow wing rising at about the limit of the first quarter. Stalked, *not double* papillae present, four pre-cloacal and four post-cloacal. About 0.12 mm. behind the cloaca the body terminates in a blunt point. Conspicuous caudal alae extend about 0.028 mm. beyond the body margin.

Raphidascaris alius sp. nov.

Host: *Salvelinus fontinalis*

Material: two males

The genus *Raphidascaris* is poorly organized and some of the descriptions of its species are inadequate, if not invalid. Several species have been reported from Europe and Asia and five North American species are already known. The present form cannot be assigned to any of these species. It differs widely in relative size of structures from *R. cayugensis* Wigdor, 1918 (23), *R. brachyurus* Ward and Magath, 1917 (22), and *R. anchoviellae* Chandler, 1935 (3) and, in the possession of similar alae and spicules, from *R. canadensis* Smedley, 1923 (19). It most closely resembles the trout species *R. laurentianus* Richardson, 1937 (16) in size and conformation, but the presence of lateral alae is a significant difference; Richardson's description of the species makes no mention of such structures. The same feature differentiates this form from *R. diadonus* Thwaite, 1927 (20); and the relative length of oesophagus and appendix are very different in *R. gigi* Fujita, 1928 as described by Yamaguti (24). Significant differences in size separate it from *R. acus* Bloch, 1779 (1) and *R. chirocentri* Yamaguti, 1935 (24).

Though the material is limited these differences necessitate the creation of a new species to accommodate it. This has been designated *Raphidascaris alius* sp. nov.

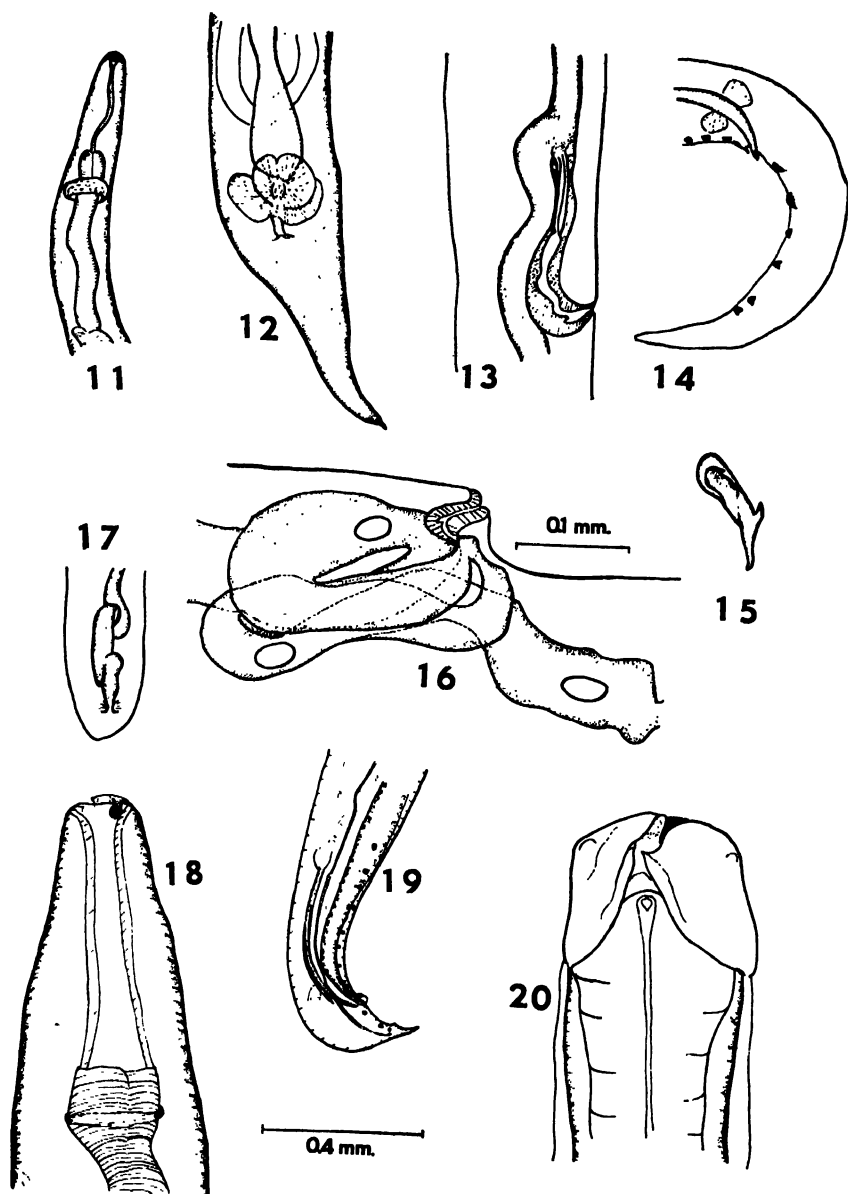
They are fairly large worms, 15.0 mm. long and 0.3 mm. wide at the base of the oesophagus and 0.4 mm. wide at the widest point. The head consists of three large lips bearing the usual papillae and conspicuous cuticular enlargements. The oesophagus is divided into a long anterior muscular portion 1.6 mm. long and a short sub-globular ventriculus 0.17 mm. long, which opens into an oesophageal appendix 1.3 mm. by 0.056 mm. and the simple intestine. The fore part of the body is curved ventrally and the tail is bent upon itself. The single testis rises just posterior to the end of the appendix, the length of the coils being greater than the width during most of its convoluted course. Just before entering the simple vas deferens, which is 0.7 mm. long, these coils are almost circular, giving a beaded appearance. Two equal and similar winged spicules are present, 0.63 mm. long and 0.028 mm. wide. They carry a terminal enlargement 0.084 by 0.112 mm. and taper to a fine point distally. The tail ends sharply 0.182 mm. behind the cloaca. On either side small pedunculate pre-cloacal papillae are present; anterior to these two or three small conspicuous elevated areas appear to mark the position of further more reduced papillae. Posterior to the cloaca three similar inconspicuous elevations probably represent the post-cloacal papillae. A conspicuous boss occupies the fore margin of the cloacal aperture. Equal lateral alae extend along the entire body length from a point just in front of the posterior extremity to the base of the lips. They are widest from the anterior limit to the region of the ventriculus.

Rhabdochona laurentiana sp. nov.

Host: *Catostomus commersonii*

Material: 5 specimens of each sex; females non-ovigerous

This species and *R. cascadilla* Wigdor, 1918, the only other reported North American species, differ in several details, but notably in the length of the oesophagus. In Wigdor's (23) species, this structure is 1/25 the length of the body; in the present material it occupies one-quarter or more of the body length. Various differences are also apparent between it and the known European and Asiatic forms. Unlike *R. accuminata* Molin, 1860 (14) and *R. denudata* Dujardin, 1845 (5), it has a well marked pre-cloacal boss and greater numbers of papillae. Its spicules are dissimilar, while those of *R. salvelini* Fujiti, 1928, are similar (6). The terminal tail spine is not found in *R. gambiana* Gendré, 1922 (7). This form lacks the lateral teeth of *R. gymnocranii* Yamaguti, 1935, but possesses vestibular rods unlike *R. girellae* Yamaguti, 1935 (24). The extensive vestibule of *R. macrolainia* Gendré, 1922 (7), and the terminal spicular barbs of *R. zaconi* Yamaguti, 1935 (24), serve as points of differentiation, as does the position of the vulva opening in *R. amago* of this author.



FIGS. 11-20. Nematodes. 11. *Rhabdochona laurentiana* sp. nov. anterior region female, from *C. commersonii*. 12. *R. laurentiana* sp. nov. posterior region female, from *C. commersonii*. 13. *R. laurentiana* sp. nov. vulvar region, female from *C. commersonii*. 14. *R. laurentiana* sp. nov. posterior end of male showing left spicule and post-cloacal papillae, from *C. commersonii*. 15. *R. laurentiana* sp. nov. right spicule, from *C. commersonii*. 16. *Cystidicoides harwoodi*, vulvar region, female, from *S. fontinalis*. 17. *C. harwoodi*, posterior end of female, from *S. fontinalis*. 18. *C. harwoodi*, anterior end of female from *S. fontinalis*. 19. *Raphidascaris alius* sp. nov. posterior end of male from *S. fontinalis*. 20. *R. alius* sp. nov. anterior end of male from *S. fontinalis*.

This form is, therefore, described as a new species, *Rhabdochona laurentiana*, the first member of the genus from *Catostomus commersonii* and the first from any Canadian host.

They are cylindrical forms, attenuated anteriorly, in which the cuticle is plain and unstriated throughout. The females are about 8.3 by 0.15 mm. with a long tail terminating in a distinct chitinized spine. The males are about 5.04 by 0.13 mm. with a long tail, but no specialized point. The lips and mouth are simple. The infundibuliform vestibule is supported by longitudinal rods which are free and pointed distally; it is about 0.08 mm. long. The oesophagus is in two distinct parts; the short anterior, muscular portion is about 0.2 mm. and usually, but not invariably, somewhat convoluted; the posterior portion is about 1.8 mm. long, occupying most of the body width where it opens into the simple and narrow intestine.

Paired, opposed genital organs occur in the female. In well developed specimens the anterior ovary rises just in front of the anterior branch of the uterus, extends to the posterior end of the oesophagus or slightly further, then curves back to join the uterus. The posterior ovary rises just behind its branch of the uterus, extends back to the anus, then returns to enter the uterus. The vulva is situated about two-thirds of the body length from the anterior end. From it the vagina runs directly posterior for about 0.2 mm. to receive the two branches of the uterus. An ovejector is present.

The male is recurved terminally. Two dissimilar spicules are present. The heavy right member, about 0.13 mm. long, is concave and equipped with backward pointing projections. The left one is simple and slender, about 0.5 mm. long, and bears a narrow wing rising in the last quarter of its length. Six conspicuous post-cloacal papillae and 12 pre-cloacal papillae are present. The last pre-cloacal members are not conspicuous. In addition to these a boss bearing a papilla-like structure is present just anterior to the cloaca. The single testis rises a short distance behind the oesophagus; it is simple and unconvoluted; no differentiation seems possible between it and the vas deferens. Neither caudal nor cephalic alae are apparent.

This was the only nematode found in *Catostomus commersonii*.

Conclusions

Though definitely limited in significance, it is interesting to note the inter-related host-parasite list. Lake trout were remarkably free from parasites, while the speckled trout were the most heavily parasitized hosts. The two other fish appear to be capable of maintaining infections of two of the parasites found in trout. *Ptychogonimus fontanus* is a strongly suckered trematode found in perch and speckled trout. The only acanthocephalid found was present in both suckers and the speckled trout.

Host Parasite List

<i>Catostomus commersonii</i>	<i>Salvelinus fontinalis</i>
<i>Glaridacris intermedius</i> sp. nov.	<i>Crepidostomum cooperi</i>
<i>Neoechinorhynchus cylindratu</i>	<i>Ptychogonimus fontanus</i>
<i>Rhabdochona laurentiana</i> sp. nov.	<i>Eubothrium salvelini</i>
<i>Cristivomer naymaycush</i>	<i>Eubothrium</i> (larval)
<i>Eubothrium</i> (larval)	Ichthyotaeniidae (larval)
Ichthyotaeniidae (larval)	<i>Neoechinorhynchus cylindratu</i>
<i>Perca flavescens</i>	<i>Cystidicoides harwoodi</i>
<i>Crepidostomum isostomum</i>	<i>Raphidascaris alius</i> sp. nov.
<i>Ptychogonimus fontanus</i>	

Acknowledgments

The assistance of the Director and staff of the Institute of Parasitology, the co-operation of the North Lake Fish and Game Club, and the privileges granted by the Department of Mines and Fisheries of the Province of Quebec were important to this work and are gratefully acknowledged.

Names of fish appearing here are those used by Hubbs (9).

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

MARCH 1940

NUMBER 3

PARACEONOGONIMUS KATSURADI SP. NOV. (TREMATODA : STRIGEIDA) FROM *LOPHODYTES CUCULLATUS* IN QUEBEC¹

BY L. L. LYSTER²

Abstract

Paraceonogonimus katsuradi sp. nov. is described from *Lophodytes cucullatus*. The sub-subfamily Prohemistomini Dubois, 1938, and the genus *Paraceonogonimus* Katsurada, 1914, must be slightly modified to accommodate it.

In the course of a search for heterophyid infections in fish-eating birds in the Lake Commandant area in the Province of Quebec, trematodes were found in the intestine of *Lophodytes cucullatus*, the hooded merganser, which upon casual examination appeared to belong to *Apophallus*. It was only when specimens were stained and mounted and the holdfast organ recognized that it became apparent that these were members of the super-super-family Strigeida Poche, 1935. The lack of marked fore- and hind-body differentiation and the pyriform shape established the relationship to the super-family Cyathocotylides Dubois, 1936, and the family Cyathocotylidae Poche, 1925. The nature of the holdfast organ, the testes, the vitellaria, and body shape were those found in the super-subfamily Prohemistominae Lutz, 1935. Though the vitellaria were not entirely limited to the post-acetabular region as in the sub-subfamily Prohemistomini, the material seemed of such obvious relationship that it was assigned to this group. The presence of an acetabulum, the lack of a specialized terminal structure, and the absence of a marked ventral pit then identify this parasite as a member of the genus *Paraceonogonimus* Katsurada, 1914. The definition of the genus describes it as a Prohemistomini in which the body is ovoid. In order that the present species may be included it is proposed that this be modified to "ovoid or pyriform."

The genus was established for the type species *P. ovatus* (3). Ransom (4), misinterpreting the nature of the holdfast organ, considered it to be a Heterophyid related to *Cryptocotyle*. Ciurea (1) somewhat tentatively had suppressed the genus earlier in favour of the strigeid genus *Prohemistomum* Odhner, 1913. Szidat (5) later showed that the genus was a valid member of the Strigeida, and Dubois (2), in his monograph, treats it as a member of the sub-subfamily Prohemistomini, placing it midway between the genera *Linstowiella* and *Prohemistomum*.

¹ Manuscript received July 22, 1939.

Contribution from the Institute of Parasitology, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

² Graduate assistant.

The sub-subfamily is defined as "Prohemistominae: vitelline follicles situated behind the acetabulum, placed near the middle of the body length, and disposed in a coronet around the holdfast organ, which is relatively well developed and hollowed by a cavity." In order that the material from the merganser may be accommodated, this definition must be slightly modified to include a species in which the vitelline follicles extend slightly anterior of the acetabulum, but are still limited to the posterior half of the body. The description of this detail would then be "Vitelline follicles situated behind the acetabulum or extending only slightly anterior to it."

The present material consists of eight specimens, of which five are egg-bearing. They are attenuate pyriform in shape, widest in the region of the holdfast organ and averaging 0.56 by 0.15 mm. in greatest measurement, with

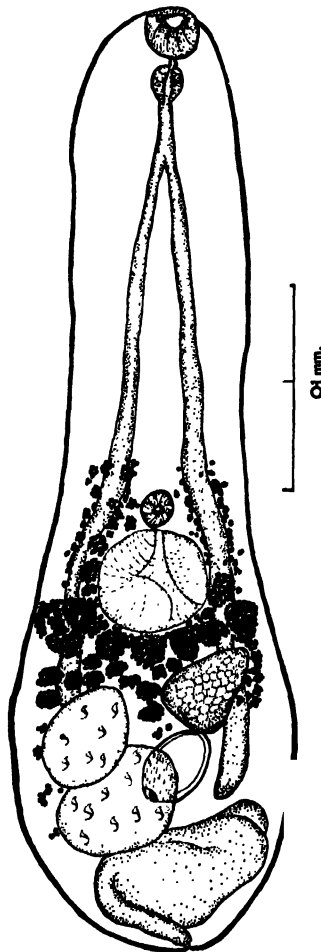


FIG 1

a maximum size of 0.63 by 0.168 mm. No cuticular spines were noted, but the cuticle was pitted as if spines might have been present before preservation. The organs are all situated in the posterior part of the body, and for this reason the post-acetabular portion is deeper than the fore part of the body, but though it is slightly hollowed ventrally there is no marked differentiation of this body region. The conspicuous but relatively weak holdfast organ, in the midline in the third quarter of the body, is 0.05 to 0.09 mm. in diameter. Anterior to it and from one-half to one-third its diameter is the acetabulum. The terminal oral sucker is 0.05 to 0.049 mm., slightly larger than the acetabulum, and is followed by a short pre-pharynx and a longitudinally ovoid pharynx about 0.05 by 0.034 mm. The oesophagus is two to four times the length of the oral sucker and opens into the crura far anterior of the acetabulum. The crura reach posteriorly to the posterior testis. The small vitellarian follicles surround the holdfast organ and are continued anterior to the acetabulum with a distinct series of follicles outlining the crura in this region; dorsal follicles are continued to the anterior region of the rear testis. The genital pore is terminal; a large and variously convoluted cirrus sac lies in the posterior extremity encroaching dorsally and laterally into the posterior testicular area, but not extending anterior of the posterior testis. The two testes are somewhat ovoid, oblique and contiguous, lying slightly to the left of the body and separated only by the cirrus sac from the posterior end. The posterior member, 0.075 by 0.08 to 0.09 mm., is larger than the other, 0.06 to 0.07 by 0.4 to 0.45 mm. The ovary, 0.05 to 0.07 mm. in greatest measurement, is roughly triangular, tending to be intermediate between the testes but on the opposite side of the body. The intra-uterine eggs, 84 by 70 μ , are always found singly.

This form differs from *P. ovatus* in several respects. It is pyriform in shape rather than ovoid, the vitellarian field is more extensive anteriorly but more limited posteriorly, and the cirrus sac is unlike that of *P. ovatus* in extent and organization. These differences serve to differentiate it from *P. ovatus*, and the material has therefore been treated as a previously undescribed species for which the name *Paraceonogonimus katsuradi* sp. nov., is proposed.

Specific Diagnosis

Paraceonogonimus: body pyriform, pre-pharynx present, oesophagus short; vitellaria surround holdfast organ dorsal and lateral to acetabulum and crura in this area, and extend dorsally to posterior testis; cirrus sac large but limited to area posterior of anterior margin of rear testis; testes oblique, ovary medial but on side of body opposite to testes.

Host: *Lophodytes cucullatus* (Hooded merganser).

Location: intestine.

Locality: Cameron's Bay, Lake Commandant, Argenteuil Co., Que.

Acknowledgments

The co-operation of the Director and staff of the Institute of Parasitology and the interest of Mr. Frank Dale, who took the mergansers, are gratefully acknowledged.

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INVESTIGATIONS ON TRICHINOSIS IN CANADA

III. ON THE INCIDENCE OF TRICHINOSIS IN GARBAGE-FED HOGS¹

BY THOMAS W. M. CAMERON²

Abstract

An examination of 995 garbage-fed hogs from Quebec, Ontario, and Manitoba, showed that only two harboured trichina larvae.

During the seasons 1937 and 1938, 2000 specimens of pork (1, 2) were examined at this Institute for the presence of trichinae. These specimens were taken at various abattoirs from Manitoba and Eastern Canada and were from *unselected* specimens. They were examined both by compression and by digestion of 10-gm. samples. They showed a general incidence of infection of 0.75%.

Recent research in the United States and elsewhere has suggested that meat-garbage fed to hogs is the main source of infection. In Canada, the Dominion Department of Agriculture requires all such garbage feeders to be licensed (and consequently to be under inspection); it further requires that all such garbage be cooked before feeding. There is no such general regulation in the United States, however. If the comparatively high incidence of trichinosis in garbage-fed hogs in that country (2) is due to this cause, it follows that, if the Department regulations in Canada are properly carried out, there should be little or no trichinosis in garbage-fed hogs in this country.

During the summer of 1939, through the co-operation of the Veterinary Director General, various abattoirs were requested to send to this Institute samples of diaphragm of hogs known to originate from hog breeders licensed to feed garbage. In this way 955 specimens were received. Apart from this, they were quite unselected. Specimens were received from Quebec, Ontario, and Manitoba only. The specimens were examined by techniques identical with those used in previous years and by the same technician who examined them in 1938. The distribution of samples is as follows.

Province	No. of samples	No. positive	Incidence
Quebec	225	2	0.88%
Ontario	283	0	0
Manitoba	487	0	0
	995	2	0.20%

¹ Manuscript received October 7, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que.; with financial assistance from the National Research Council of Canada.

² Professor, McGill University; and Director, Institute of Parasitology, Macdonald College, Que., Canada.

Only two specimens contained trichina larvae. The first of these was one of a lot of 17 hogs, the second one of a lot of 10 hogs. Both lots originated in close proximity to the city of Montreal.

In the previous two years' investigation, 2,000 hogs were examined. These were unselected and consequently may have contained both garbage- and non-garbage-fed hogs. While not strictly comparable with this year's examination, the cumulative figures for the three years are as follows:

Province	No. of samples	No. positive	Incidence
Manitoba	1,253	5	0.39%
Ontario	834	3	0.36%
Quebec	725	8	1.10%
Maritime Prov.	157	1	0.65%
Saskatchewan	7	—	—
Unknown	19	—	—
Total	2,995	17	0.57%

The total of 2,995 hogs, of which at least a third are known to be fed on garbage, shows an incidence of 0.57%. The known garbage-fed hogs alone show an incidence of 0.20%.

It would appear legitimate to draw the following tentative conclusions from this survey.

1. The incidence of trichinosis in garbage-fed hogs in the United States is estimated at about 5%. The much lower figure found in this survey suggests that the cooking of garbage is a reasonably sound method of preventing the condition, even although an occasional case occurs. It is probable that such a case is due to insufficient cooking, although an extraneous source of infection cannot be excluded.

2. The general incidence of porcine trichinosis in Eastern Canada appears to be considerably lower than in the United States.

3. The comparatively high figures for the Province of Quebec are probably misleading. Two cases have been definitely traced to garbage-fed hogs. All the remaining six cases (reported in 1937) were recovered from one lot of seven samples received from a single centre and it is not unreasonable to assume that they represent a single infection; this infection is possibly traceable to garbage-fed hogs also although there is no information available.

4. The figures for the Maritime Provinces are based on too few samples to be reliable. It is more probable that a figure close to 0.4% (as shown by Ontario and Manitoba) is a more representative one. Much larger numbers are necessary, however, before this can be accepted. It is probable that even this low figure could be further reduced by a more strict application of the regulations regarding garbage cooking.

Acknowledgments

The examinations were made by Mr. Angus MacMillan, and the author has to thank Dr. A. E. Cameron, Veterinary Director General of Canada, for his co-operation in this survey, and Drs. G. A. Rose, Ed. Dufresne, Ed. Grandmaison, H. H. Anderson, R. D. Boast, and I. W. Purdy, for the actual collection of the specimens. These Inspectors' assistance was invaluable and all specimens received were in excellent condition.

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REPTANT DECAPOD CRUSTACEA OF THE WEST COASTS OF VANCOUVER AND QUEEN CHARLOTTE ISLANDS, BRITISH COLUMBIA¹

BY JOSEPHINE F. L. HART²

Abstract

The range, size, local distribution, and notes on 44 species of reptant decapod crustaceans taken in British Columbia coastal waters are presented. A summary of records from the literature has been compiled, and the distribution of the species as a result of the peculiar configuration of the coast line is illustrated from these data.

The present paper records the range, size, local distribution, and notes on 44 species of reptant decapod crustaceans taken in the waters off the coast of British Columbia by collectors from the Pacific Biological Station. With these have been combined the records of the same species presented in the literature. Since the coast of British Columbia is probably unique in that it includes wide stretches of coast exposed to the open Pacific as well as equally large areas bordering sheltered inland passages, a summary of the records from the literature for the reptant decapods has been compiled from which an attempt is made to illustrate the difference in the distribution of the species as a result of the peculiar configuration of the coast line.

The material available consists of some 750 specimens, representing 44 species of reptant decapod Crustacea. The larger part of the collection was made in the summer of 1934 by Mr. E. G. Hart, while he was serving as Biologist for the Pacific Biological Station on the C.G.S. "Wm. J. Stewart" on the west coast of Vancouver Island. The remainder was obtained by Dr. C. McLean Fraser in 1935 when he was acting in a similar capacity on the west coasts of the Queen Charlotte Islands. The areas represented by the collections comprise the middle part of the west coast of Vancouver Island and the southern two-thirds of the west coasts of the Queen Charlotte Islands.

A few dredge hauls and shore collections in these areas, as well as along the remainder of the west coasts of Vancouver and Queen Charlotte Islands, were made by the United States Fisheries Steamer "Albatross" from 1888 to 1891 (1, 12-15). Collections were also made from the Queen Charlotte Islands by Dr. G. M. Dawson (Smith, 17), from Clayoquot, west coast of Vancouver Island, by Newcombe (10), from Ucluelet, Barkley Sound, west coast of Vancouver Island, by Macoun (Rathbun, 13-15), and from Clayoquot Sound by Spencer (18). A few records of species are also to be found in the lists of acquisitions of Crustacea in the Annual Reports of the British Columbia Provincial Museum (3). Only one-half the number of species in the present collection has been listed in these papers as occurring in localities on the west

¹ *Manuscript received October 9, 1939.*

Contribution from the Pacific Biological Station, Nanaimo, B.C.

² *Volunteer Investigator, Pacific Biological Station.*

coasts of Vancouver and Queen Charlotte Islands. The distribution records for the east coast of Vancouver Island, Victoria, and Friday Harbour, Washington, and northern British Columbia, are from papers listed in the references, with the exception of a few species that have been collected in the vicinity of Departure Bay by the author, but that have not been previously recorded.

One difficulty encountered is that a number of species have been reported in distribution records simply as occurring in British Columbia, since many of the early workers did not give specific localities, designating their specimens only as from "Vancouver Island", "Gulf of Georgia", "Queen Charlotte Island shore", etc. In view of the area included and of the great ecological differences involved, this is not at all satisfactory. The author has therefore endeavoured to be more specific, particularly in the case of species whose northern limit lies in British Columbia. When species are found on both east and west coasts of Vancouver Island, both localities are given as the northern range. This may be somewhat cumbersome, but is of value to local workers, and may be simplified with future collecting, when the species will perhaps be found to encircle the Island. Another difficulty is that two species have been confused, or have been recorded as one. Thus the early records for species of the genera *Petrolisthes*, *Chionoecetes*, and the rare species of *Cancer* cannot be used.

As will be seen by reference to the charts and tables included in this paper, the area concerned on the west coast of Vancouver Island was divided into a number of somewhat arbitrary regions, each of which contains consecutively numbered collection stations. The Barkley Sound area was designated as area 2211; Clayoquot Sound as 2221, 2222, and 2223; Nootka Sound as 2231 and 2232; and Esperanza Inlet as 2241, 2242, and 2243. The west coast of the Queen Charlotte Islands was considered as a unit and numbered area 3500.

In the following list, 11 species, whose northern limit lies in British Columbia, have been found farther north than previously recorded. The limit of distribution of those species that extend north and south of British Columbia has been obtained mainly from Rathbun's papers. The author is indebted to the Smithsonian Institution for the records of specimens of *Pagurus hemphillii* (Benedict) and *Orthopagurus minimus* (Holmes) from British Columbia in the collections of the United States National Museum, Washington, D.C. The size given is the length, or the length and breadth, of the carapace. The limits are the sizes of specimens in the collection.

Upogebia pugettensis (Dana)

Range. Southeastern Alaska to San Quentin Bay, Lower California.

Size. ♀ 14–35 mm. ♂ 10–37 mm.

Local distribution.	Esperanza Inlet area,	2241–13,	2 ♀	2 ♂
	Queen Charlotte Islands,	3529,		2 ♂

TABLE I
DATA CONCERNING COLLECTION OF MATERIAL

Station No.	Latitude N	Longitude W	Date	Method of collection
2211- 1	48°39'	125°46'	May 8, 1934	T 70 m.
2221- 3	49°07'45"	126°31'30"	15,	T 130 m.
8	49°10'30"	126°13'30"	14,	T 56 m.
11	48°52'	126°11'	1,	T 101 m.
14	49°46'15"	125°57'	July 5,	T 77 m.
16	48°59'45"	126°07'45"	May 1,	T 73 m.
17	49°00'15"	126°07'15"	1,	T 73 m.
18	49°02'45"	126°02'30"	1,	T 60 m.
20	49°00'15"	125°44'30"	11,	T 18-21 m.
22	49°19'45"	126°21'45"	16,	T 50 m.
25	49°14'	126°20'	23,	T 64 m.
26	49°11'	126°11'	23,	T 45 m.
27	49°10'30"	126°10'15"	23,	T 50 m.
28	49°11'30"	126°39'	June 5,	T 118 m.
2222- 4	49°09'45"	126°00'	11,	S
6	49°15'15"	126°07'	12,	S
2223- 1	49°28'30"	126°23'45"	May 29,	S
3	49°22'15"	126°28'45"	June 1,	S
2231- 6	49°08'30"	126°49'	5,	T 170-180 m.
9	49°10'	126°43'	5,	T 155 m.
10	49°13'30"	126°43'	5,	T 113 m.
13	49°21'	126°43'30"	9,	T 55 m.
14	49°23'45"	126°34'15"	May 31,	S
15	49°23'45"	126°34'15"	June 2,	S
16	49°34'45"	126°40'15"	13,	S
17	49°36'45"	126°48'15"	14,	S
18-I	49°33'30"	126°38'45"	18,	B 89 m.
19	49°31'15"	126°42'45"	19,	T 73 m.
20	49°36'45"	126°49'	26,	S
28	49°36'	126°43'15"	July 9,	S
29	49°36'	126°43'15"	10,	S
30	49°37'15"	126°49'30"	11,	S
31	49°22'15"	126°54'	12,	T 75-90 m.
32	49°31'45"	126°34'45"	13,	S
33	49°22'30"	126°55'	17,	T 137 m.
34	49°30'	126°51'	17,	T 95 m.
2241- 8	49°39'15"	126°57'15"	18,	B 45 m.
9	49°37'15"	126°50'30"	June 16,	S
11	49°40'45"	126°53'30"	27,	S
12	49°51'	127°06'15"	28,	S
13	49°45'	126°59'	July 14,	S
15	49°38'45"	127°00'	19,	T 73 m.
2242- 2	49°51'15"	127°04'45"	June 25,	S
3	49°44'	126°57'	July 24,	S
4	49°44'	126°57'	25,	S
5	49°44'	126°57'	26,	S
6	49°48'	126°58'15"	27,	S
3507	West side Louscoone Bay, 1 mile from entrance		June 4, 1935	S
3513	Head of Big Inlet		6,	T
3515	Rennell Sound between Gospel Is. and south shore.		10,	T
3516	Northeast of Marble Is.		11,	T
3519	Tasu Hbr., north shore 2 miles from entrance		14,	T
3521	Near Rose Hbr., Houston-Stewart Channel		18,	S
3525	Rennell Sound		24,	T
3526	9½ mi. south of Marble Is.		26,	B

TABLE I—*Concluded*DATA CONCERNING COLLECTION OF MATERIAL—*Concluded*

Station No.	Latitude N	Longitude W	Date	Method of collection
3527	Near south shore, Tasu Hbr.		27,	T
3529	Canoe Passage, Skidegate Ch.		30,	S
3530	West side, eastern Skidegate Narrows		July 1,	S
3532	Skidegate Channel, near western Narrows		3,	S
3536	Flamingo Hbr.		13,	From kelp
3537	South shore, Houston-Stewart Channel		16,	S
3539	Half-tide rock, entrance Flamingo Hbr.		17,	S
3540	Rocks, entrance to Big Inlet		18,	S

T—trawl; S—shore; B—bottom.

Munida quadrispina Benedict

Range. Sitka, Alaska to Los Coronados, Lower California.

Size. ♀ 10–23 mm. ♂ 9.5–73 mm.

Local distribution. Barkley Sound area, 2211–1, 1 ♂
 Nootka Sound area, 2231–6, 1 ♀ 1 ♂
 2231–10, 1 ♀ 7 ♂
 Queen Charlotte Islands, 3516, 1 ♂
 3525, 2 ♀

Petrolisthes eriomerus Stimpson

Range. Flamingo Inlet, Queen Charlotte Islands, B.C., to Lower California.

Size. ♀ 6 × 6–12.5 × 12.5 mm. ♂ 7.5 × 6.5–14.5 × 14 mm.

Local distribution. Clayoquot Sound area, 2221–25, 1 ♀
 2223–3, 1 ♀
 Nootka Sound area, 2231–30, 2 ♀
 Esperanza Inlet area, 2241–13, 1 ♂
 2242–3, 1 ♀
 2242–4, 3 ♂
 Queen Charlotte Islands, 3507, 4 ♀ 1 ♂
 3539, 1 ♂

Remarks. This species has been previously recorded in British Columbia only from Victoria and Nanaimo, and thus its occurrence on the west coast of Vancouver Island and the southwest of the Queen Charlotte Islands constitutes a considerable extension of its range.

Petrolisthes cin tipes (Randall)

Range. Rose Harbour, Houston-Stewart Channel, Queen Charlotte Islands, B.C., to Gulf of California.

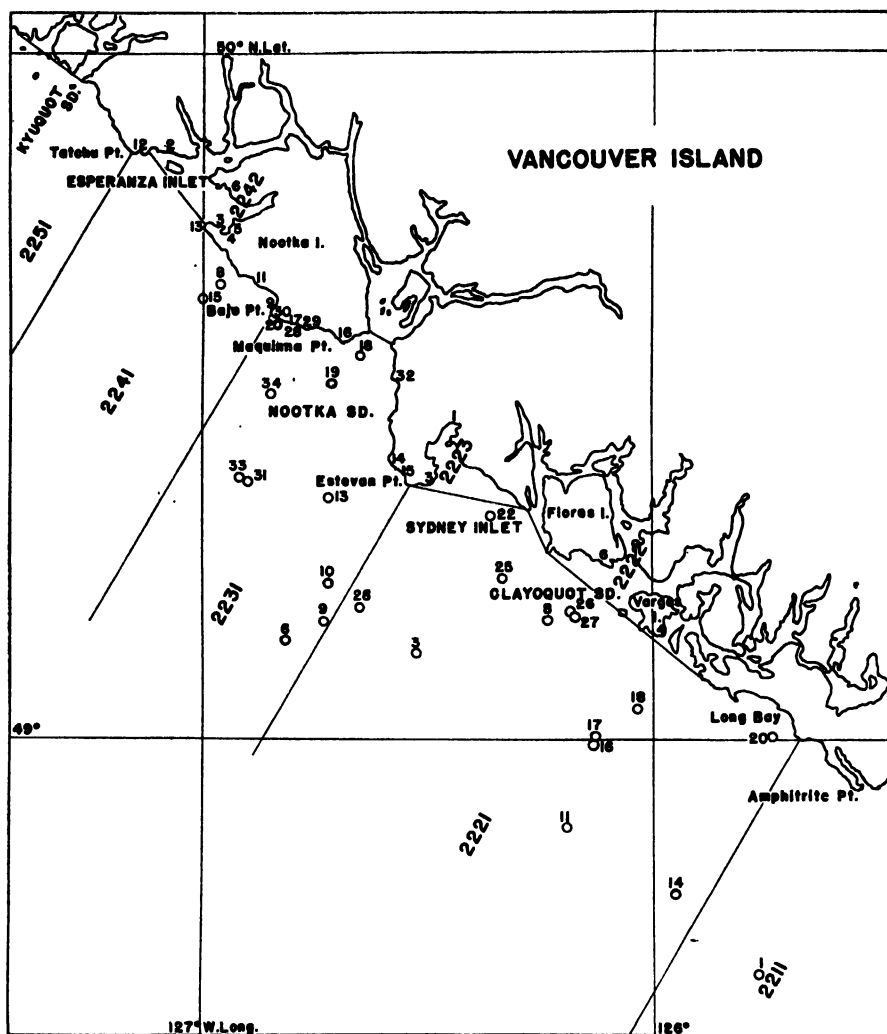


FIG. 1. Showing locations of the following Stations: 2211-1; 2221-3, 8, 11, 14, 16, 17, 18, 20, 22, 25, 26, 27, 28; 2222-4, 6; 2223-1, 3; 2231-6, 9, 10, 13, 14, 15, 16, 17, 18-1, 19, 20, 28, 29, 30, 31, 32, 33, 34; 2241-8, 9, 11, 12, 13, 15; 2242-2, 3, 4, 5, 6.

Size. ♀ 10.5 × 10.5–24 × 24 mm. ♂ 10 × 9–17 × 16 mm.

Local distribution.	Clayoquot Sound area,	2222-6,	1 ♀
	Nootka Sound area,	2231-14,	1 ♀ 1 ♂
		2231-30,	1 ♀
	Esperanza Inlet area,	2242-3,	1 ♀
	Queen Charlotte Islands,	3507,	1 ♂
		3521,	3 ♂

Remarks. This species was recorded by the early naturalists from the east and west coasts of Vancouver Island. *P. eriomerus* Stimpson was not mentioned, and as Taylor (21) states that *P. cinctipes* was common at Nanaimo, where only *P. eriomerus* is now found, it is not possible to use

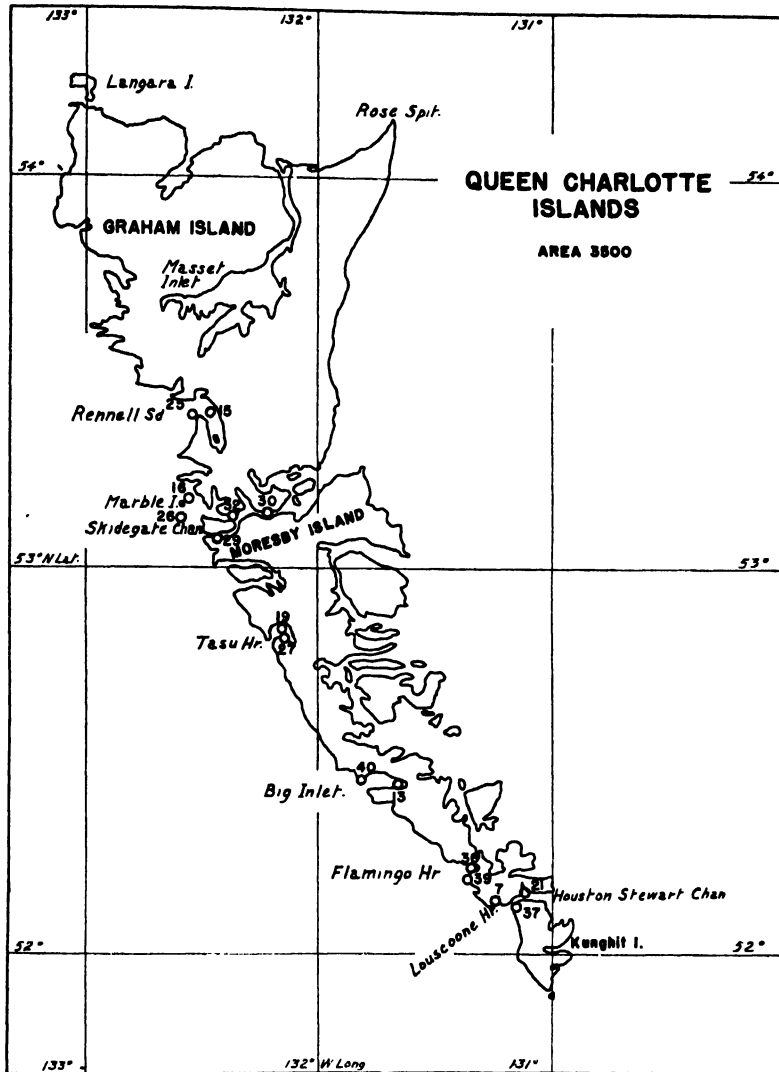


FIG. 2. Showing locations of the following stations in the 3500 area: 07, 13, 15, 16, 19, 21, 25, 26-B, 27, 29, 30, 32, 36, 37, 39, 40.

the early records. Since there has been no recent record of *P. cinclipes* from the east coast of Vancouver Island or from Puget Sound, it would appear that this species occurs in British Columbia only on the west coast of Vancouver Island and the Queen Charlotte Islands. Its northern known limit is extended considerably by this collection.

Pachycheles rudis Stimpson

Range. Kodiak, Alaska, to San Diego, California.

Size. ♀ 8 × 8–19.5 × 20.5 mm. ♂ 3.5 × 3.5–17 × 18 mm.

Local distribution.	Clayoquot Sound area,	2223-1,	1 ♀	1 ♂
	Nootka Sound area,	2231-16,	1 ♀	2 ♂
		2231-20,	2 ♀	
		2231-29,		1 ♂
		2231-30,	4 ♀	
	Esperanza Inlet area,	2242-3,	7 ♀	2 ♂
		2242-4,	2 ♀	6 ♂

Remarks. This species exhibits a great variety of colour combinations. Most are reddish in formalin, but some have a brown or purple tinge, and are mottled grey and fawn. Some looked bleached and the chelae are white.

Pachycheles pubescens Holmes

Range. Nuchatlitz Inlet, Esperanza Inlet, Vancouver Island, B.C., to Monterey Bay, California.

Size. ♂ 13 × 13.5 mm.

Local distribution. Esperanza Inlet area, 2242-4, 1 ♂

Remarks. This is the first British Columbia record of this species and is a considerable northern extension of its range. The carapace is brick red in formalin, except for the rostrum and posterior margin, which is grey mottled with brown. The granules on the chelae are white and the inner margin is reddish.

Pagurus alaskensis (Benedict)

Range. Siberia and Alaskan coast of Bering Sea to Oregon.

Size. ♀ 7.5-10 mm. ♂ 5.5-14 mm.

Local distribution.	Clayoquot Sound area,	2221-27,		1 ♂
	Nootka Sound area,	2231-6,	1 ♀	
		2231-13,	1 ♀	
		2231-19,		1 ♂
		2231-31,		3 ♂
	Esperanza Inlet area,	2241-15,	1 ♀	1 ♂

Pagurus aleuticus (Benedict)

Range. Bering Sea (Pribilof Islands) to Oregon.

Size. ♀ 7 mm. ♂ 7-10 mm.

Local distribution.	Nootka Sound area,	2231-9,		1 ♂
		2231-33,	1 ♀	1 ♂

Pagurus ochotensis (Brandt)

Range. Unalaska to San Diego, California.

Size. ♀ 4-11 mm. ♂ 5-14 mm.

Local distribution.	Barkley Sound area,	2211-1,		2 ♂
	Clayoquot Sound area,	2221-8,	1 ♀	1 ♂
		2221-20,	1 ♀	
		2221-26,		2 ♂

Nootka Sound area,	2231-19,	2 ♀	1 ♂
	2231-31,	2 ♀	1 ♂
Esperanza Inlet area,	2241-15,		1 ♂
Queen Charlotte Islands,	3519,	12 ♀	12 ♂
	3527,	1 ♀	1 ♂

Pagurus brandti (Benedict)

Range. Bering Sea (latitude of Pribilof Islands) southward to Oregon.

Size. ♀ 4 mm. ♂ 4 mm.

Local distribution. Queen Charlotte Islands, 3519, 1 ♀ 1 ♂

Pagurus dalli (Benedict)

Range. Bering Sea to Oregon.

Size. ♀ 3-4 mm. ♂ 6 mm.

Local distribution. Clayoquot Sound area, 2221-8, 1 ♂
Nootka Sound area, 2231-19, 5 ♀

Pagurus confragosus (Benedict)

Range. Bering Sea (latitude of Pribilof Islands) to mouth of the Columbia River, Oregon.

Size. ♀ 10 mm.

Local distribution. Nootka Sound area, 2231-9, 1 ♀

Remarks. This species has not been recorded previously from British Columbia, although found in waters north and south of this region. The colour in formalin is light, mottled with pinkish-brown dorsally except on the chelae and the centre of the hard part of the carapace. There is a greater depth of colour on either side of the distal joints of the walking legs.

Pagurus kennerlyi (Stimpson)

Range. Aleutian Islands to Washington.

Size. ♀ 3.5-11 mm. ♂ 3.5-5 mm.

Local distribution. Barkley Sound area, 2211-1, 1 ♂
Clayoquot Sound area, 2221-8, 1 ♀
2221-17, 2 ♂
2221-18, 1 ♀

Pagurus beringanus (Benedict)

Range. Bering Sea (latitude of Nunivak) to Monterey, California.

Size. ♀ 5 mm. ♂ 3.5-5 mm.

Local distribution. Clayoquot Sound area, 2221-18, 6 ♀ 1 ♂
Queen Charlotte Islands, 3519, 1 ♂
3527, 1 ♀

Pagurus setosus (Benedict)

Range. Kodiak, Alaska, to Santa Cruz Islands, California.

Size. ♀ 4–6 mm. ♂ 4–11 mm.

Local distribution.	Clayoquot Sound area,	2221–18,	1 ♀	
	Nootka Sound area,	2231–9,		2 ♂
	Queen Charlotte Islands,	3519,		1 ♂
		3525,	1 ♀	
		3530,		1 ♂

Pagurus hirsutiusculus (Dana)

Range. Aleutian Islands to San Diego, California. Japan. Siberia.

Size. ♀ 4–7 mm. ♂ 4.5–15 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		2 ♂
	Nootka Sound area,	2231–9,		1 ♂
		2231–28,	2 ♀	2 ♂
	Esperanza Inlet area,	2242–3,		1 ♂
	Queen Charlotte Islands,	3529,	2 ♀	
		3537,	1 ♀	

Pagurus granosimanus (Stimpson)

Range. Unalaska to Ensenada, Lower California.

Size. ♀ 6–8 mm. ♂ 7.5–9.5 mm.

Local distribution.	Clayoquot Sound area,	2222–6,	1 ♀	
	Nootka Sound area,	2231–15,		1 ♂
		2231–17,		1 ♂
		2231–20,	1 ♀	1 ♂
		2231–29,		1 ♂
	Queen Charlotte Islands,	3507,	1 ♀	1 ♂

Pagurus hemphillii (Benedict)

Range. Houston-Stewart Channel, Queen Charlotte Islands, B.C., to Monterey, California.

Size. ♀ 5–10 mm. ♂ 4–14 mm.

Local distribution.	Nootka Sound area,	2231–17,		2 ♂
	Esperanza Inlet area,	2241–12,		1 ♂
		2241–13,		2 ♂
		2242–3,	1 ♀	1 ♂
		2242–4,		1 ♂
	Queen Charlotte Islands,	3507,	1 ♀	1 ♂
		3537,		2 ♂

Remarks. These records are all north of any previously recorded specimens (those in the United States National Museum are from Barkley Sound). In formalin, the colour is dark red with white carapace dorsally, and the tips of the dactyls of all the legs and the propodi of the chelipeds are light coloured.

Paguristes turgidus (Stimpson)

Range. Rennell Sound, Queen Charlotte Islands, B.C., to San Diego, California.

Size. ♀ 4–13.5 mm. ♂ 4–22 mm.

Local distribution.	Barkley Sound area,	2211–1,		1 ♂
	Clayoquot Sound area,	2221–3,	1 ♀	2 ♂
		2221–14,	1 ♀	
	Nootka Sound area,	2231–6,	2 ♀	
		2231–9,	1 ♀	
		2231–10,	1 ♀	
		2231–13,	1 ♀	1 ♂
		2231–19,		1 ♂
		2231–31,	15 ♀	14 ♂
		2231–33,	5 ♀	15 ♂
		2231–34,	1 ♀	
	Esperanza Inlet area,	2241–15,	2 ♀	1 ♂
	Queen Charlotte Islands,	3515,	3 ♀	2 ♂
		3527,		1 ♂

Remarks. This species has not been recorded from the northern points previously.

Orthopagurus schmitti (Stevens)

Range. Esperanza Inlet, and Departure Bay, Vancouver Island, B.C., to Puget Sound, Washington.

Size. ♀ 3–6 mm.

Local distribution.	Barkley Sound area,	2211–1,	1 ♀
	Esperanza Inlet area,	2242–4,	6 ♀

Remarks. This is the first record of this species from outside waters, as it has been found previously only in Puget Sound and the Gulf of Georgia, B.C. It occupies empty *Serpulid* tubes.

Orthopagurus minimus (Holmes)

Range. Skidegate, Queen Charlotte Islands, B.C., to San Francisco, California.

Size. ♀ 3–5.5 mm. ♂ 4–5 mm.

Local distribution.	Queen Charlotte Islands,	3519,	1 ♂
		3527,	4 ♀ 2 ♂

Remarks. Schmitt (16) gave the range as Queen Charlotte Sound instead of Queen Charlotte Islands. That this is an error has been verified for me by the United States National Museum. This species occupies empty *Dentalium* shells. The large chelae and the cephalothorax are deep red and the rest is straw-coloured in formalin.

Oedignathus inermis (Stimpson)

Range. Unalaska to Pacific Grove, California. Japan.

Size. ♀ 5 × 4–17 × 16 mm. ♂ 4 × 3.5–30 × 25 mm.

Local distribution.	Clayoquot Sound area,	2222-4,		1 ♂
		2222-6,	1 ♀	
	Nootka Sound area,	2231-14,		2 ♂
		2231-15,		1 ♂
		2231-30,	1 ♀	
		2231-32,	1 ♀	
	Esperanza Inlet area,	2241-12,		1 ♂
		2242-3,	1 ♀	
		2242-4,		1 ♂
	Queen Charlotte Islands,	3539,	8 ♀	7 ♂
		3540,		1 ♂

Remarks. The coloration, in formalin, is usually red-brown with violet granules on the large cheliped. The claws of the walking legs are black, with deep yellow bands on the distal part of the propodi.

Hapalogaster mertensii Brandt

Range. Atka, Aleutians, eastward and southward to Puget Sound.

Size. ♀ 10 × 11-17 × 19 mm. ♂ 15 × 16-22 × 24 mm.

Local distribution. Queen Charlotte Islands, 3521, 2 ♀ 2 ♂

Remarks. The absence of this species from the west coast of Vancouver Island, is perhaps significant, but no definite statement can be made without more extensive distribution records.

Cryptolithodes sitchensis Brandt

Range. Sitka, Alaska, to Pacific Grove, California.

Size. ♂ 7 × 10-36 × 52 mm.

Local distribution.	Clayoquot Sound area,	2223-3,	1 ♂
	Nootka Sound area,	2231-17,	1 ♂
	Esperanza Inlet area,	2242-4,	2 ♂

Remarks. One specimen in formalin is brick-red dorsally, and white, tan and red, ventrally; another pearl-grey with a symmetrical pattern of dark red dorsally and with the dorsal side of the chelipeds and legs tan; another a dirty white; and the fourth, grey etched with black, and periopods brown dorsally.

Lopholithodes foraminatus (Stimpson)

Range. Clayoquot Sound and Nanoose Bay, Vancouver Island, to San Diego, California.

Size. ♀ 110 × 130-120 × 140 mm.

Local distribution. Clayoquot Sound area, 2221-3, 3 ♀

Remarks. The northern limit for this species is usually given as Victoria, although Taylor (21) reports seeing it at Nanaimo. There are specimens from Nanoose Bay, north of Nanaimo, in the Museum of the Pacific Biological Station, Nanaimo, B.C.

Oregonia gracilis Dana

Range. Bering Sea to Monterey, California. Japan.

Size. ♀ 11 × 7–35 × 19 mm. ♂ 7 × 5–40 × 20 mm.

Local distribution.	Barkley Sound area,	2211–1,	2 ♀	1 ♂
	Clayoquot Sound area,	2221–3,		1 ♂
		2221–8,	10 ♀	8 ♂
		2221–16,	34 ♀	31 ♂
		2221–17,	11 ♀	19 ♂
		2221–20,	3 ♀	9 ♂
		2221–22,	1 ♀	2 ♂
		2221–26,	7 ♀	3 ♂
		2221–27,		1 ♂
		2221–28,	1 ♀	2 ♂
		2223–1,	1 ♀	
	Nootka Sound area,	2231–13,	3 ♀	
		2231–19,	2 ♀	2 ♂
		2231–29,	1 ♀	1 ♂
		2231–31,	5 ♀	1 ♂
		2231–34,	4 ♀	7 ♂
	Esperanza Inlet area,	2241–8,		1 ♂
		2242–6,		1 ♂
	Queen Charlotte Islands,	3519,		1 ♂
		3527,		1 ♂

Pugettia producta Randall

Range. Houston-Stewart Channel, Queen Charlotte Islands, to Santa Rosalia Bay, Lower California.

Size. ♀ 13 × 9–50 × 42 mm. ♂ 25 × 21–40 × 28 mm.

Local distribution.	Clayoquot Sound area,	2222–4,		1 ♂
	Nootka Sound area,	2231–28,	2 ♀	
		2231–32,		1 ♂
	Esperanza Inlet area,	2241–13,	1 ♀	
	Queen Charlotte Islands,	3521,		1 ♂

Remarks. This species has been previously recorded only from Vancouver Island southward.

Pugettia gracilis Dana

Range. Western extremity of the Aleutian Islands eastward and southward to Mendocino, California.

Size. ♀ 15 × 11–35 × 28 mm. ♂ 9 × 8–35 × 28 mm.

Local distribution.	Clayoquot Sound area,	2222–6,	1 ♀	
	Nootka Sound area,	2231–20,		2 ♂
		2231–28,		2 ♂
		2231–30,	1 ♀	
		2231–32,		1 ♂

Esperanza Inlet area,	2241-11,	1 ♂
	2242-3,	1 ♀
	2242-4,	1 ♀
Queen Charlotte Islands,	3507,	5 ♀
	3521,	4 ♀
	3539,	3 ♀

Pugettia richii Dana

Range. Esperanza Inlet, Vancouver Island, B.C., to San Diego, California.

Size. ♀ 9 × 6-33 × 26.5 mm. ♂ 9.5 × 7-44 × 36 mm.

Local distribution. Clayoquot Sound area,	2223-1,	1 ♂
	2223-3,	2 ♂
Nootka Sound area,	2231-14,	1 ♀
	2231-17,	1 ♂
	2231-20,	1 ♀
	2231-28,	3 ♀
	2231-30,	3 ♀
Esperanza Inlet area,	2241-11,	1 ♂
	2241-12,	1 ♀
	2242-3,	6 ♀
	2242-4,	1 ♀
	2242-5,	1 ♂

Remarks. The northern limit of range of this species is extended by the present collection. There is considerable variation in colour, from red to brown, and the legs are usually banded with light and dark colour. A number of the specimens are covered with encrustations of bryozoans, hydroids, algae, etc.

Mimulus foliatus Stimpson

Range. Unalaska to Monterey Bay, California. Mexico.

Size. ♀ 11 × 11.5-23 × 24.5 mm. ♂ 11 × 10-35 × 39 mm.

Local distribution. Clayoquot Sound area,	2223-1,	1 ♂
	2223-3,	1 ♂
Nootka Sound area,	2231-20,	3 ♂
	2231-28,	1 ♂
	2231-30,	2 ♀
Esperanza Inlet area,	2241-12,	1 ♀
	2242-3,	1 ♀
	2242-4,	2 ♀
	2242-5,	1 ♂
Queen Charlotte Islands,	3507,	1 ♂
	3521,	1 ♀

Remarks. There is considerable variation in the coloration, but the presence of a distinct V on the carapace seems to be fairly consistent. There are light coloured bands between the hepatic and lateral spines, which join

medially over the cardiac region. The predominant colour is red or red-brown, with a white V and red and white striped legs. The chelae are lighter in colour than the carapace.

Scyra acutifrons Dana

Range. Kodiak, Alaska, to San Diego, California.

Size. ♀ 13 × 9.5–36 × 26 mm. ♂ 10 × 8–46 × 35 mm.

Local distribution.	Nootka Sound area,	2231-16,	1 ♀	
		2231-20,	1 ♀	3 ♂
		2231-28,	3 ♀	1 ♂
		2231-29,	1 ♀	
		2231-30,	4 ♀	1 ♂
		2231-32,		1 ♂
	Esperanza Inlet area,	2241-12,	2 ♀	2 ♂
		2242-3,	4 ♀	
		2242-4,	3 ♀	7 ♂
		2242-5,	2 ♀	
	Queen Charlotte Islands,	3537,		1 ♂

Remarks. The carapace of the larger specimens is usually covered with an encrustation of sponges, tunicates, barnacles, etc. The tubercles are rose-coloured, and the legs banded with red and white.

Chorilia longipes Dana

Range. Shumagin Bank and Kodiak, Alaska, to San Diego, California. Japan.

Size. ♀ 8 × 4.5–34 × 20 mm. ♂ 7 × 4–21 × 11 mm.

Local distribution.	Clayoquot Sound area,	2221-16,	5 ♀	
		2221-17,	3 ♀	2 ♂
	Nootka Sound area,	2231-31,	1 ♀	
	Queen Charlotte Islands,	3526,		1 ♂
		3527,	2 ♀	1 ♂

Hyas lyratus Dana

Range. Bering Sea to Admiralty Inlet, Washington.

Size. ♀ 13 × 8.5 mm.

Local distribution.	Nootka Sound area,	2231-31,	1 ♀
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Cancer productus Randall

Range. Kodiak, Alaska, to Laguna Beach, California.

Size. ♀ 37 × 57–55 × 85 mm. ♂ 10 × 11–27 × 42 mm.

Local distribution.	Clayoquot Sound area,	2222-4,		1 ♂
		2223-1,		1 ♂
		2223-3,		1 ♂
		2231-30,	1 ♀	
	Nootka Sound area,	2231-32,		1 ♂

Esperanza Inlet area,	2241-13,	1 ♀
	2242-3,	1 ♂
	2242-4,	1 ♀

Remarks. The usual variety of colour in the small specimens is found: pure white; bands of brown and white; stripes of red and white; brown striped. The larger specimens are a uniform red.

Cancer magister Dana

Range. Unalaska to Monterey Bay, California.

Size. ♀ 35 × 50 mm. megalopae.

Local distribution.	Clayoquot Sound area,	2222-4,	1 ♀
	Nootka Sound area,	2231-28,	2 megalopae
	Esperanza Inlet area,	2241-9,	2 megalopae

Cancer branneri Rathbun

Range. Granite Cove, Port Althorp, Alaska, to Santa Catalina Island, California.

Size. ♀ 13 × 17-28 × 39 mm. ♂ 4.5 × 5.5-35 × 54 mm.

Local distribution.	Barkley Sound area,	2211-1,	2 ♀
	Clayoquot Sound area,	2221-17,	1 ♂
		2221-20,	2 ♀ 3 ♂
		2221-26,	12 ♀ 7 ♂
		2221-27,	1 ♀ 2 ♂
	Esperanza Inlet area,	2241-8,	2 ♂

Cancer oregonensis (Dana)

Range. Pribilof Island and Rat Island, Alaska, to Santa Barbara, California.

Size. ♀ 10 × 14-17 × 21.5 mm. ♂ 4.5 × 5-9.5 × 12 mm.

Local distribution.	Clayoquot Sound area,	2221-25,	1 ♀
		2222-6,	1 ♀
	Nootka Sound area,	2231-14,	1 ♂
	Esperanza Inlet area,	2241-13,	1 ♀
		2242-3,	2 ♂
		2242-4,	3 young

Telmessus cheiragonus (Tilesius)

Range. Bering Sea to California.

Size. ♂ 7 × 7 mm.

Local distribution.	Queen Charlotte Islands,	3536,	1 ♂
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Lophopanopeus bellus (Stimpson)

Range. Prince William Sound, Alaska, to Monterey, California.

Size. ♀ 8 × 11-15 × 22 mm. ♂ 13 × 19-20.5 × 30 mm.

Local distribution.	Nootka Sound area,	2231-20,	1 ♀	1 ♂
		2231-29,		2 ♂
		2231-30,	6 ♀	
	Esperanza Inlet area,	2241-12,		1 ♂
		2241-13,		1 ♂
		2242-3,	2 ♀	1 ♂
	Queen Charlotte Islands,	3507,		1 ♂

Pinnixa faba (Dana)

Range. Prince of Wales Island, Alaska, to Humboldt Bay, California.

Size. ♀ 5 × 9 mm. ♂ 6.5 × 11 mm.

Local distribution.	Nootka Sound area,	2231-14,		1 ♂
	Esperanza Inlet area,	2242-6,	1 ♀	

Remarks. The male is tan with a white mottled branchial region and the female is white, mottled with brown.

Pinnixa littoralis Holmes

Range. Sitka, Alaska, to San Diego, California.

Size. ♀ 11.5 × 18.5-16 × 20 mm.

Local distribution.	Esperanza Inlet area,	2242-3,	1 ♀	
	Queen Charlotte Islands,	3532,	1 ♀	

Remarks. The "liver" shows yellow and the ovary orange through the transparent carapace.

Pinnixa occidentalis Rathbun

Range. Unalaska to Magdalena Bay, Lower California.

Size. ♀ 2 × 4-2.5 × 5 mm. ♂ 2 × 5-3 × 6.5 mm.

Local distribution.	Queen Charlotte Islands,	3513,	12 ♀	9 ♂
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Pinnixa schmitti Rathbun

Range. Port Levasheff, Alaska, to San Francisco Bay, California.

Size. ♀ 2.5 × 4.5 mm. ♂ 2.5 × 5 mm.

Local distribution.	Esperanza Inlet area,	2242-6,	1 ♀	1 ♂
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Scleroplax granulata Rathbun

Range. Esperanza Inlet and Departure Bay, Vancouver Island, B.C., to Ensenada, Lower California.

Size. ♀ 4.5 × 6-5 × 6 mm.

Local distribution.	Esperanza Inlet,	2242-6,	3 ♀	
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Of the 44 species in the collection, two, *Pachycheles pubescens* Holmes and *Pagurus confragosus* (Benedict), have not been previously recorded from British Columbia. Five species found in the Queen Charlotte Islands collec-

tion are not represented in the Vancouver Island collection, but as all of these range southward, it is probable that they occur here also. *Hemigrapsus nudus* (Dana) and *H. oregonensis* (Dana), the common shore crabs, do not occur in the west coast of Vancouver Island collection. Two explanations may be advanced: First, both species require the protection of gravel or loose rocks, which condition apparently is not commonly met with on that rugged, surf-washed shore; and second, at low tide the shore crabs are sufficiently high up on the beach to be entirely missed when collections are made near low water line. That these species do occur in this region there can be little doubt, as the larvae were present in the zooplankton taken from the same area. Moreover, Spencer (18) reports them abundant throughout Clayoquot Sound. Fraser did not collect these two species but identified them in the field at a number of Queen Charlotte Islands shore stations. He also identified, but did not collect, *Cancer magister* Dana, *C. productus* Randall, and *C. oregonensis* (Dana).

Table II is a compilation of the records of the 69 species of reptant Decapoda that have been found in British Columbia. The coast has been divided into eight regions: southern Vancouver Island (Victoria, B.C., and Friday Harbour, Washington); the east coast of Vancouver Island and the corresponding mainland coast; four areas on the west coast of Vancouver Island; the Queen Charlotte Islands; Queen Charlotte Sound, and northeast coasts. Records from published works are indicated by *p*, unpublished personal records by *H* and the present collection by *X*.

TABLE II
OCCURRENCE OF REPTANT DECAPODA IN BRITISH COLUMBIA

	Gulf of Georgia	Victoria, B.C., and Friday Hbr., Washing- ton	Barkley Sound	Clayo- quot Sound	Nootka Sound	Esper- anza Inlet	Queen Char- lotte Islands	Queen Char- lotte Sound and north
<i>Munida quadrispina</i> Benedict	<i>p</i>	<i>p</i>	<i>X</i>		<i>X</i>		<i>Xp</i>	<i>p</i>
<i>Petrolisthes eriomerus</i> Stimpson	<i>p</i>	<i>p</i>		<i>X</i>	<i>X</i>	<i>X</i>	<i>X</i>	
<i>Petrolisthes cinctipes</i> (Randall)				<i>Xp</i>	<i>X</i>	<i>X</i>	<i>X</i>	
<i>Pachycheles rudis</i> Stimpson		<i>p</i>	<i>p</i>	<i>Xp</i>	<i>X</i>	<i>X</i>		
<i>Pachycheles pubescens</i> Holmes						<i>X</i>		
<i>Upogebia pugettensis</i> (Dana)	<i>p</i>	<i>p</i>		<i>p</i>		<i>X</i>	<i>Xp</i>	
<i>Callinassa californiensis</i> Dana	<i>p</i>	<i>p</i>		<i>p</i>				
<i>Callinassa gigas</i> Dana		<i>p</i>						
<i>Pagurus alaskensis</i> (Benedict)	<i>p</i>	<i>p</i>		<i>X</i>	<i>X</i>	<i>X</i>		
<i>Pagurus aleuticus</i> (Benedict)	<i>p</i>	<i>p</i>			<i>X</i>			<i>p</i>
<i>Pagurus ochotensis</i> Brandt	<i>p</i>	<i>p</i>	<i>Xp</i>	<i>X</i>	<i>X</i>	<i>X</i>	<i>X</i>	
<i>Pagurus brandti</i> (Benedict)	<i>H</i>	<i>p</i>					<i>X</i>	<i>p</i>
<i>Pagurus dalli</i> (Benedict)	<i>p</i>	<i>p</i>		<i>X</i>	<i>X</i>			
<i>Pagurus confragosus</i> (Benedict)					<i>X</i>			
<i>Pagurus cornutus</i> (Benedict)								<i>p</i>
<i>Pagurus gilli</i> (Benedict)	<i>H</i>	<i>p</i>						
<i>Pagurus beringanus</i> (Benedict)	<i>p</i>	<i>p</i>		<i>X</i>			<i>X</i>	
<i>Pagurus kennerlyi</i> (Stimpson)	<i>p</i>	<i>p</i>	<i>X</i>	<i>X</i>				
<i>Pagurus selosus</i> (Benedict)	<i>p</i>	<i>p</i>		<i>X</i>	<i>X</i>		<i>X</i>	
<i>Pagurus hirsutiusculus</i> (Dana)	<i>p</i>	<i>p</i>		<i>X</i>	<i>X</i>	<i>X</i>	<i>X</i>	

TABLE II—*Concluded*OCCURRENCE OF REPTANT DECAPODA IN BRITISH COLUMBIA—*Concluded*

	Gulf of Georgia	Victoria, B.C., and Friday Hbr., Washington	Barkley Sound	Clayoquot Sound	Nootka Sound	Esperanza Inlet	Queen Charlotte Islands	Queen Charlotte Sound and north
<i>Pagurus granosimanus</i> (Stimpson)	p	p		X	X		X	p
<i>Pagurus hemphillii</i> (Benedict)			p		X	X	X	
<i>Pagurus tenuimanus</i> (Dana)	H	p						p
<i>Pagurus splendescens</i> Owen	p	p						
<i>Paguristes turgidus</i> (Stimpson)	p	p	X	X	X		X	p
<i>Parapagurus mertensii</i> (Brandt)					p			
<i>Orthopagurus minimus</i> (Holmes)							Xp	
<i>Orthopagurus schmitti</i> (Stevens)	p	p	X			X		
<i>Hapalogaster mertensii</i> Brandt	II	p					X	
<i>Oedignathus inermis</i> (Stimpson)				X	X	X	Xp	
<i>Acantholithodes hispidus</i> (Stimpson)	p	p					p	
<i>Placetron wosnessenskii</i> Schallfeew		p					p	
<i>Cryptolithodes sitchensis</i> Brandt		p		X	Xp	X	p	
<i>Cryptolithodes typicus</i> Brandt	p	p						p
<i>Lopholithodes foraminatus</i> (Stimpson)	p	p		X				
<i>Lopholithodes mandtii</i> Brandt	p	p	p					
<i>Rhinolithodes wosnessenskii</i> Brandt		p						p
<i>Paralithodes rostrifalcatus</i> McKay								p
<i>Paralithodes camtschatica</i> Tilesius							p	
<i>Paralomis multispina</i> (Benedict)							p	
<i>Phyllolithodes papillosus</i> Brandt	p	p						
<i>Oregonia gracilis</i> Dana	p	p	X	Xp	X	X	Xp	p
<i>Pugettia producta</i> Randall	p	p	p	Xp	X	X	X	p
<i>Pugettia gracilis</i> Dana	p	p	p	Xp	X	X	Xp	p
<i>Pugettia richii</i> Dana		p	p	Xp	X	X		
<i>Mimulus foliatus</i> Stimpson			p	Xp	X	X	X	
<i>Scyra acutifrons</i> Dana	p	p	p		X	X	Xp	p
<i>Chorilia longipes</i> Dana	p	p		X	X		X	p
<i>Chionoecetes bairdi</i> Rathbun	p							p
<i>Chionoecetes angulatus</i> Rathbun							p	
<i>Hys lyratus</i> Dana	p	p		p	X			p
<i>Telmessus cheiragonus</i> (Tilesius)	p	p		p			Xp	p
<i>Cancer productus</i> Randall	p	p	p	Xp	X	X	p	p
<i>Cancer branneri</i> Rathbun	II		Xp	X		X		
<i>Cancer gracilis</i> Dana	p	p		p				
<i>Cancer magister</i> Dana	p	p		Xp	X	X	p	p
<i>Cancer oregonensis</i> (Dana)	p	p		X	X	X	p	p
<i>Lophopunopeus bellus</i> (Stimpson)	p	p		p	X	X	X	
<i>Pinnotherees pugetlensis</i> Holmes	p	p						
<i>Pinnotherees taylora</i> Rathbun	p		p					
<i>Fabia subquadrata</i> Dana	p	p					p	
<i>Pinnixa faba</i> (Dana)	p	p		p	X	X		
<i>Pinnixa littoralis</i> Holmes	p	p	p			X	X	
<i>Pinnixa occidentalis</i> Rathbun	p							p
<i>Pinnixa schmitti</i> Rathbun	H	p				X		
<i>Pinnixa tubicola</i> Holmes	p	p						p
<i>Scleroplax granulata</i> Rathbun	p	p				X		
<i>Hemigrapsus nudus</i> (Dana)	p	p		p				p
<i>Hemigrapsus oregonensis</i> (Dana)	p	p		p				p

p — previously recorded; H — in author's collection; X — in 1934 and 1935 collections.

It is only the shore and shallow water forms that one would expect to be appreciably influenced by the different conditions found on the exposed west coast as compared with the sheltered waters of the Gulf of Georgia, which borders the east coast. Of these conditions the temperature of the water is probably the chief limiting factor, as was suggested for the difference in littoral distribution of *Pagurus beringanus* (6). The surface water temperatures of the west and south coasts of Vancouver Island are consistently lower than those of the Gulf of Georgia. As a result, at Victoria, this species may be collected at low tide, while to obtain specimens of a similar size at Nanaimo it is necessary to dredge in deeper water.

Of the 69 species recorded from British Columbia, 63 have been found in the waters surrounding Vancouver Island. More than half of these may be obtained at low tide. The remainder are normally taken only by dredging and thus are often rather poorly represented in collections. Two of these species, *Chionoëcetes bairdi* Rathbun and *Pinnixa occidentalis* Rathbun, have been found only on the east coast of Vancouver Island, while four others, *Pagurus confragosus* (Benedict), *Parapagurus mertensii* (Brandt), *Placetreron wosnessenskii* Schallfeew, and *Rhinolithodes wosnessenskii* Brandt have been found only on the south or west coasts. Since none of these species has been taken in large numbers, or often, the range will probably be extended by future collecting. Nine shore and shallow water forms have been found only on the south or west coasts. Of these, *Callianassa gigas* Dana and *Pachycheles pubescens* Holmes have been rarely taken, but *Petrolisthes cinctipes* (Randall), *Pachycheles rudis* Stimpson, *Pagurus hemphillii* (Benedict), *Oedignathus inermis* (Stimpson), *Cryptolithodes sitchensis* Brandt, *Pugettia richii* Dana, and *Mimulus foliatus* Stimpson seem to be fairly abundant on the west coast of Vancouver Island, and thus would appear to require the cold waters and rugged conditions associated with proximity to the open ocean.

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APOPHALLUS IMPERATOR SP. NOV., A HETEROPHYID ENCYSTED IN TROUT, WITH A CONTRIBUTION TO ITS LIFE HISTORY¹

BY L. L. Lyster²

Abstract

Apophallus imperator sp. nov., (Heterophyidae : Trematoda) is described and figured. It is a potential human parasite of which the metacercarial stage is encysted in *Salvelinus fontinalis* in the Province of Quebec. The other hosts are unknown; experimentally, it becomes adult in cats and pigeons. The significance of the genus *Apophallus* and related genera is reviewed and the systematics of *Apophallus* discussed. Organization of the gonotyl is the only feature accepted as uniformly distinctive of this genus and modifications in that structure are traced and recommended for their significance in specific diagnoses.

Introduction

In the course of a survey of parasitism in trout during the summer of 1938, an interesting condition was reported among Speckled Trout (*Salvelinus fontinalis*) taken from parts of Lake St. Bernard in the Province of Quebec. The fins and the skin under the scales in a specimen sent from this area were disfigured by numerous black spots. Microscopic examination showed that this was caused by the encysted metacercaria of some trematode. To establish the identity of the parasite, part of the trout was given to a cat that had never previously been fed fish. On post-mortem examination of the cat 12 days later, several minute flukes, easily recognizable as members of the family Heterophyidae, were recovered.

Recent studies have illustrated the economic interest of the Heterophyidae. The lack of host specificity among many genera of the family makes it important to fur-bearing animals and wild life as well as to man. Human heterophyidiasis has been known for many years, and Africa, Garcia, and de Leon in the Philippines have demonstrated a potential high pathogenicity in human infections (2, 4). Not only is the intestinal mucosa parasitized but the eggs of the parasite may be carried by the blood stream to the heart and the central nervous system with serious effects. Cameron (8) recently recovered unidentified heterophyid eggs from a patient in a local hospital, the first recorded human case in North America.

Various marine and freshwater fish are known to be heterophyid vectors. One of the carriers of *Metagonimus* in Japan is a salmonid fish, but no infection in North American species of these fish has been reported.

Trout carrying the metacercariae were easily obtained from the southern end of Lake Commandant, Argenteuil Co., Quebec. The condition has been

¹ Manuscript received July 22, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council and the Provincial (Quebec) Scientific Commission.

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known to exist there for several years and all experimental material came from there.

Description (Fig. 1)

Body linguiform, pyriform, or discoid, covered with spines in parallel rows, most numerous from oral sucker to intestinal bifurcation, very few posterior to testes and none at extreme anterior tip. Oral sucker subterminal, 0.063 by 0.056 mm. Pre-pharynx very short, usually entirely hidden behind the oral sucker. Pharynx longitudinally ovoid. Oesophagus long, weak, extending for one-half length of body, or somewhat less. Caeca larger than oesophagus, extending to well behind testes. Acetabulum relatively well developed and large, 0.077 by 0.070 mm., medial and posterior to intestinal furcation. Genital sinus opening anterior to ventral sucker; two gonotyls dorsal and anterior to acetabulum, 0.021 by 0.027 mm., narrowly separated. Seminal vesicle large structure dorsal and posterior to acetabulum in midline, curving ventrally and laterally, narrower terminal portion; dorsal and lateral portions sac-like and separated by constrictions. Ovary pyriform, 0.026 to 0.031 mm. by 0.056 to 0.077 mm., on left side of body nearer to ventral sucker than to testes. Seminal receptacle large, globular, anterior to ovary. Testes two, 0.057 to 0.063 mm. by 0.112 to 0.126 mm., triangular in shape, usually oblique but sometimes almost side by side, or almost tandem; right member anterior; posterior member removed from end by about its own diameter. Vasa efferentia continue in vas deferens to join seminal vesicle left of acetabulum. Uterus short, few turns, contains 5 to 20 large brown eggs, 0.014 to 0.019 mm. by 0.033 to 0.035 mm., reaches genital opening from left side of acetabulum. Common vitelline ducts rise at level of anterior testis. Vitellaria rise at level of gonotyl, extend to posterior end in a narrow limited band of scattered follicles, extending towards median field immediately behind testes and at level of common vitelline duct, but rarely meeting. Excretory bladder S-shaped with arms to pharyngeal area.

VARIATIONS IN MORPHOLOGY

All adult specimens were treated in the same way. The intestinal mucosa of the experimental animal was scraped into saline and sedimented with the addition of a little sodium bicarbonate. The living flukes were pipetted into cold fixative (glacial acetic formalin alcohol) and shaken. They were preserved in a mixture of 5% glycerine in 70% alcohol, to which had been added 5% formalin, then washed in distilled water and dilute alcohol and stained in acid alum carmine. In spite of this, there is a wide range of body shape (Figs. 4-7). Those from the pigeon are more uniformly pyriform, those from the cat usually ovoid, constricted in the region of the ventral sucker.

Two specimens show vitellaria extending anteriorly as far as the bifurcation of the oesophagus. In all others, the vitellaria are restricted to an area behind the gonotyl. In one of the two variant specimens the ventral sucker has been displaced antero-laterally and lies up the right side of the body near

the base of the oesophagus. In both, the testes are almost tandem and removed posteriorly. The status of these forms is difficult to determine. Important features have been obscured in mounting; one might well be *Apophallus venustus* but the other seems definitely a malformation of the present species.

Identification

This form, obviously a heterophyid, is also recognizable as a member of the genus *Apophallus sensu lato*. This group is poorly understood. In order that identification may be clear some discussion of the related genera that include *Apophallus* is necessary here. It is limited as far as possible to *Apophallus* and the genera that have been confused with it.

History

The genus *Apophallus* was created in 1909 by Luhe (18) with *Distomum muhlingi* Jagerskiold, 1899, from *Larus ridibundus* as type species; it was incorrectly described as *Distomum lingua* by Creplin in 1898.

In 1919 Skrjabin and Lindtrop (23) erected the genus *Rossicotrema* with *R. donicum* as type species, from the cat and dog, distinguishing it from *Apophallus* because of the condition of the vitellaria and testes.

Without reference to that genus, Ransom in America created the genus *Cotylophallus* in 1920 (22) based on the arrangement of the vitellaria; it contained two species, *C. venustus* from the cat, dog, and wolf, and *C. similis* from *Phoca vitulina*, those also being distinguished by vitellaria arrangement. In the same paper he described *A. brevis* from *Larus delawarensis*.

All subsequent authors have treated *Cotylophallus* as a synonym of *Rossicotrema*.

In 1924, Szidat (25) described *Apophallus major* from *Larus fuscus*, basing his new species only on absolute measurements.

In 1931, Price (20) described *A. crami* from *Larus californicus*, in which the vitellaria were limited to the post-ovarian region. A year later (21) he created *A. zalophi* from *Zalophus californicus*, differing widely from the type in organization of the vitellaria, development of the pre-pharynx and other details. The species *A. americanus* was described on two immature specimens from the stomach of fish in 1932 by Van Cleave and Mueller (27). The specimens were apparently ectopic forms and the specific identification is based on features now known to be variable. The species *americanus* may well be valid, but in reporting it the authors imply that they are creating it largely to avoid confusion until further material is available.

In 1935, Africa (3) described the species *eccentricus* which he referred to *Apophallus*, though it differed in several details usually considered super-generic and mainly in the position of the gonotyl.

Thus, two closely related genera, *Apophallus* and *Rossicotrema*, containing nine species between them, have been introduced into the literature. These Ciurea (9) placed together in his sub-family Apophallinae, because of common

characters of the acetabulo-genital complex (notably that the genital sinus opens anterior to the acetabulum).

Witenberg (28), reviewing the family, placed *Apophallus* and *Rossicotrema* together with *Cryptocotyle* and *Tocotrema* (in which the genital sinus opens posterior to the acetabulum) in the new tribe *Cryptocotylea*, and in the sub-family Heterophyinae which included the sub-families Metagoniminae, Apophallinae, and Cryptocotylinae as understood by Ciurea. He considered the arrangement of the acetabulo-genital complex of sub-generic importance and differentiated the tribe solely on the anterior extent of the vitellaria. This division would remove *A. crami*, in spite of its very obvious relationships, to his tribe Heterophyinae, where there is no genus to accommodate it. Witenberg refused to accept comparison of vitellarian fields for separating species. He reduced *R. donicum*, *R. (-Cotylophallus) similis* and *R. (-Cotylophallus) venustum* to synonymy, and transferred *A. brevis* to this genus, making it a synonym of *R. donicum*. He discounted the specific importance of size and made *A. major* a synonym of *A. muhlingi*.

In 1930 (29), in amending his previous monograph in several details, he made the genus *Rossicotrema* a synonym of *Tocotrema* Looss, 1899. He considered the condition and position of the acetabulum and position and number of gonotyls of specific interest only. Stunkard (24), in describing the life history of the type species, *Tocotrema lingua*, did much to establish its congenerity with *Cryptocotyle* Luhe, 1899, a relationship that had previously been accepted by all workers with two exceptions (Ciurea (10) and Linton (16)).

Price (20), in 1931, considered that the acetabulo-genital complex furnished generic characters. Thus, he regarded *Rossicotrema* as distinct from *Tocotrema* (and, therefore, from *Cryptocotyle*) but indistinguishable from *Apophallus*. In the last genus he recognized *muhlingi*, *crami*, *donicus* (= *venustus* and *similis*), and *brevis*, as valid species, all separable on features of the vitellaria. Ciurea (10) did not agree that *Apophallus* and *Rossicotrema* could be synonyms, because of variations in body shape, arrangement of testes, and development of metacercariae. He concurred, however, in the differentiation of *Tocotrema* and *Rossicotrema*, which he assigned to the sub-families Cryptocotylinae and Apophallinae respectively.

The sub-family Cryptocotylinae had been created by Luhe (18), 1909, to contain the genera *Cryptocotyle* and *Scaphanocephalus*. To these Ciurea added the genera *Tocotrema* and *Ciureana*. The sub-family is characterized mainly by the reduced condition of the acetabulum and genital sucker and the position of the genital opening posterior to the acetabulum.

The sub-family Apophallinae, created by Ciurea (9), is based on the extent of development of the genital sucker and acetabulum and the position of the genital opening, anterior to the acetabulum. He has placed the genera *Apophallus*, *Rossicotrema*, *Euryhormis*, and *Pricetrema* in it. The genus *Pricetrema* was erected at this time by Ciurea to accommodate *A. zalophi* Price, 1931. It is separated because of the length of the oesophagus, the situation of the testes, and the course of the seminal vesicle and uterus.

Poche (19) created the genus *Euryhormis* to include *Distomum squamula* Rudolphi, 1819, but incompletely described it. Recently Baer (6), 1931, has made a more definite contribution to our knowledge of the morphology of this form. He points out the unique organization of the acetabulo-genital complex. The genital sucker, situated anterior to the acetabulum, consists of a *single* papillae-like muscular structure attached anteriorly. Very recently a description and life history of a new species, *Euryhormis monorchis*, has been published by Ameel (5). In spite of its single testis, he placed it in the sub-family Heterophyinae *sensu* Witenberg, but his observations refute rather more than support the inclusion of this genus with *Apophallus* in the restricted sub-family Apophallinae *sensu* Ciurea.

Cameron (7) in a discussion on the family in 1936 accepted the synonymy of *Apophallus* and *Rossicotrema* but discounted the importance of body shape and size as a diagnostic character. He accorded only limited value to testicular arrangement for separating species but he acknowledged the importance of the vitellarian field. By this criterion he was able to distinguish *A. venustus* from *A. donicus*, *muhlingi*, and *brevis*, but not from *A. similis*, which he concluded to be a synonym.

It is apparent from this brief review that a great deal of confusion has resulted in these genera because of lack of agreement as to what should constitute generic and specific features.

The Genus

The genus *Apophallus* has previously been recorded under various synonyms. Price and Cameron consider *Rossicotrema* synonymous with it. *Rossicotrema* has been accepted as synonymous with *Cotylophallus*, and Witenberg considered it as a synonym of *Cryptocotyle* by various workers. *Pricetrema* was created by Ciurea to accommodate a species originally placed in *Apophallus*.

Some convention must be accepted to facilitate classification of this group. The obvious variation in the associated genera under discussion is in the structure of the acetabulo-genital complex. This feature has been variously considered of specific, generic, and super-generic significance.

Should the specific valuation be accepted, as recommended by Witenberg (28), a confusing group of synonymous designations results. No logical series of generic and specific characteristics can be traced. The variation in the complex seems to divide this part of the family into two distinct groups as represented by the genera *Cryptocotyle* and *Apophallus*. In the first a *single* gonotyl guards the genital opening *posterior* to the acetabulum. In the second, a *pair* of gonotyls guards the genital opening *anterior* to the acetabulum.

At the moment it is difficult to evaluate fully the true significance of this division, whether generic or super-generic. Two complementary factors must be considered:

1. The position of the genital opening.
2. The number of gonotyls.

The species *A. eccentricus* is unique because of the lateral opening of its genital pore. If valid, this species may represent a series of undiscovered forms which would be intermediate between the two genera and make *Apophallus* a synonym of *Cryptocotyle*. However, re-study will probably illustrate its closer relationship to *Stictodora* than to *Apophallus*.

A single gonotyl seems a constant feature among the genera in which the genital opening is posterior. With two exceptions all species with the anterior genital opening have two gonotyls. These exceptions, *Euryhormis squamula* and *E. monorchis*, are of poorly understood phylogeny. They may represent a parallel organization which would place *Euryhormis* in the same sub-family as *Apophallus* (as Ciurea (10) has done); they may be a continuation of the *Apophallus* type of development and therefore be congeneric with it; or, as available evidence suggests, they may have developed distinctly from *Apophallus* and thus bear no relationship to this genus. Present evidence, then, does not support super-generic or specific evaluations of these major features of differentiation, although in theory either may be correct. Following Price (20) and Cameron (7), generic rank of this character seems most practicable.

Genus *Cryptocotyle*

Reduced acetabulum, single gonotyl, posterior genital opening.

This genus is of no further interest in the present discussion.

Genus *Apophallus* (= *Prictrema*, *Cotylophallus*, and *Rossicotrema* (not synonymous with *Tocotrema*).

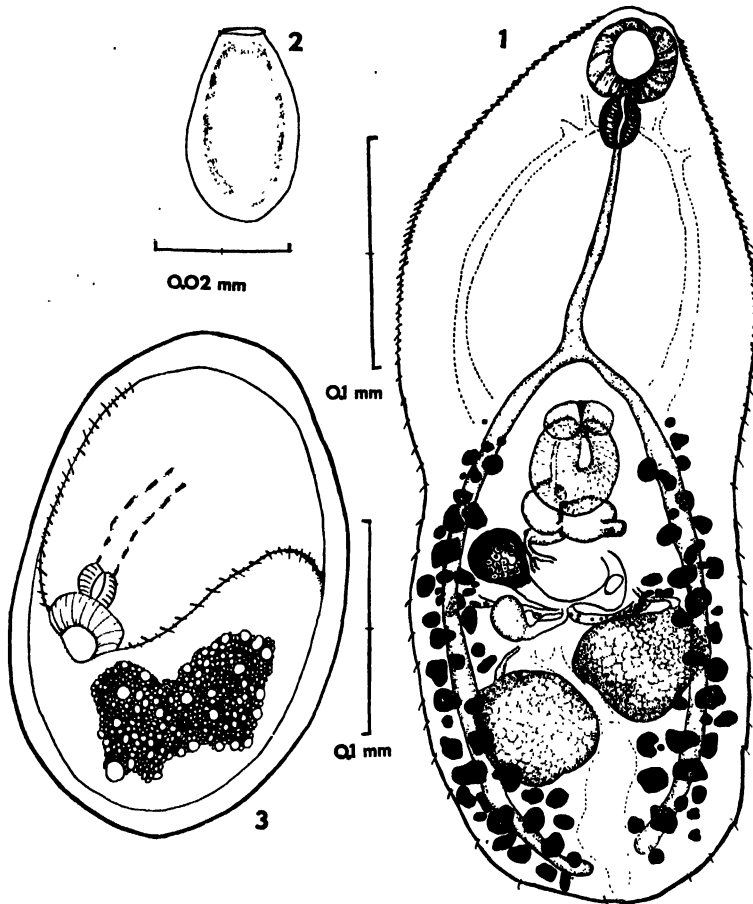
More distinct acetabulum, paired gonotyls, anterior genital opening.

This genus is essentially the same as Ciurea's sub-family Apophallinae, with the exclusion of the genus *Euryhormis*. The present species is assigned to it.

The Species

Less clearly indicated are the specific characters; Ciurea, Africa, and Cameron and the present paper have proved the inconstancy of shape and size. The relative position of testes is variable within some species, more constant in others, but never suitable as a specific feature. Many authors have accepted the extent and arrangement of the vitellaria for diagnosing species. By this feature *A. venustus*, in which the vitellaria reach the crural furcation, is separable from *A. donicus* and *A. brevis*, in which the follicles do not exceed the gonotyls. In studying *A. donicus* (from the collection of Dr. Ciurea) and *A. brevis* (from the collection of Dr. Cameron) I find that the anterior limit of the vitellaria is constant but does not provide differences for complete differentiation of these species. From the same material it is seen that there is considerable variation in the medial follicles within each species. In *A. brevis*, the vitellaria meet behind the testes, but in front of these organs they are usually limited to the lateral fields. In a few specimens, however, they almost meet anterior to the ventral sucker. In *A. donicus*, the same condition may be noted, but in reverse order. The vitellaria usually meet in the midline anterior to the ventral sucker, but in a few specimens they

are limited to the lateral fields in this area. (Cameron's statement that the vitellaria did *not* reach "the ventral sucker" in these forms is probably a *lapsus*, intended for "the intestinal bifurcation".) These variations demonstrate the fact that the arrangement of the vitellaria is not acceptable for identifying species when considered alone. Further less variable characters must be found.

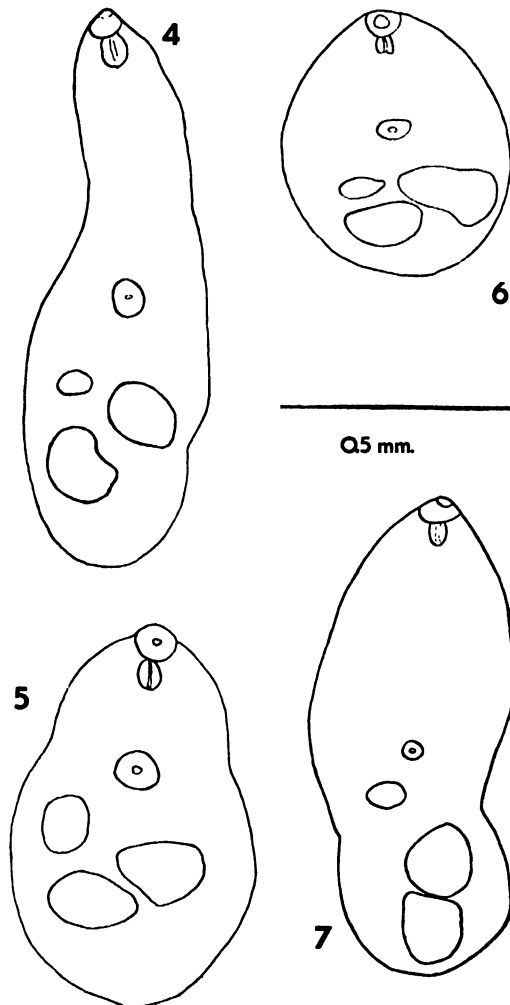


FIGS. 1-3. *Apophallus imperator* sp. nov. FIG. 1. Adult from experimental cat. FIG. 2. Eggs from faeces of same host. FIG. 3. Encysted metacercaria from trout.

Absolute measurements have not been accepted, but comparative measurements and morphology, especially of the more heavily muscled structures, should be valuable. The more strongly organized and least variable structures are the seminal vesicle, the oral sucker, and the acetabulo-genital complex. Of these, modification in the seminal vesicle is seen upon study to be a super-specific factor, and the oral sucker is important when considered in comparison with the ventral sucker, but does not afford a complete range of variations. Of the three, the acetabulo-genital complex seems likely to be the most satisfactory structure upon which to base criteria. The genital

sucker is most subject to the evolutionary changes that characterize the family to the extent that it has been assigned a special term "gonotyl." The genus, too, is identified by the condition of the remnant of this organ. Each species then might show a distinct development of the important structure.

The acetabulo-genital complex was studied in the three species of the genus *Apophallus* that have been confused in North American reports, *donicus*, *venustus*, and *brevis*, and in the present form and found to be separable. In *A. venustus* the ventral sucker is strongest; anterior to it is the genital structure, weakly muscled but illustrating distinctly its affinity to the true genital sucker of other trematodes, and lacking the extensive papillae-like construction of the organ as found in the other species of the genus (Fig. 8).



FIGS. 4-7. *Apophallus imperator* sp. nov. FIGS. 4 AND 5. Outlines of adults from pigeon. FIGS. 6 AND 7. Outlines of adults from cat.

In the remaining species the ventral sucker is less strongly muscled, and the genital structure is less weak. In *A. donicus* the papilla-like development is also somewhat limited, but not to the same extent as in *A. venustus* (Fig. 9). In *A. brevis* and the present form the structure consists of two distinct and strong bodies with little apparent relation to a primitive sucker. In the former species these gonotyls are large and extend laterally well beyond the acetabulum (Fig. 10), while in the latter they are roughly unilateral with this organ (Fig. 11).

Thus, two types of variable development are found in the remnant of the genital sucker in these species:

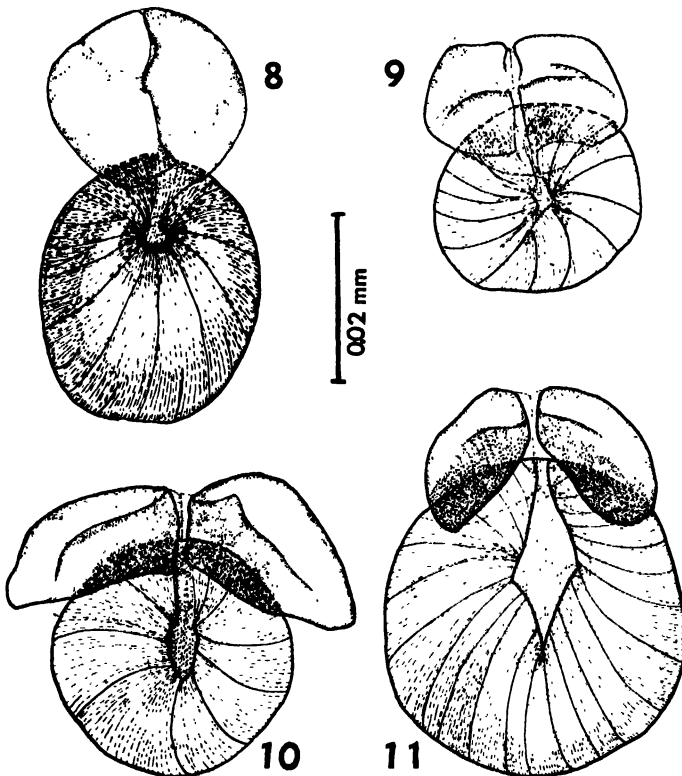
1. Papillae-like, far removed from progenital type—

brevis—gonotyls large, extend laterally beyond margin of acetabulum.
 present species—gonotyls not so large, do not extend past lateral margins.

2. Not papillae-like, showing affinity to true genital sucker—

venustus—gonotyls compound globular structures.
donicus—gonotyls closely associated but more distinct than in *venustus*.

The remaining species are already easily distinguishable, with the exception of *muhlingi*.



FIGS. 8–11. Acetabulum and gonotyl of *Apophallus* spp. FIG. 8. *A. venustus*. FIG. 9. *A. donicus*. FIG. 10. *A. brevis*. FIG. 11. *A. imperator*.

A. muhlingi and the present form have some otherwise unique features in common. In each the vitellaria reach just to the level of the gonotyls and never extend medially except behind and between the testes. Similarly the acetabulum in each is usually slightly larger than the oral sucker. Though no specimens of *A. muhlingi* were available for comparison, Ciurea's careful discussion on it provides details for differentiation (10). The eggs in *A. muhlingi* are much broader than in the present form, though of approximately the same length. Of greater value is the nature of the gonotyls. He describes these organs as being symmetrical hemispheres with a diameter of 0.017 mm. His illustration shows a pair of such structures with the complete diameter parallel to the axis of the body. In the present form the gonotyls are 0.021 mm. in this measurement and 0.021 mm. transversely. Thus, the gonotyls of the two forms vary in size, shape, and direction of greatest measurement, though they are the most nearly alike of the species discussed.

Though it is closely related to *muhlingi* and *brevis*, the writer is thus able to distinguish this trout-carried member of the genus and considers it to represent a hitherto undescribed species, for which the designation *imperator* is proposed (from the locality in which it was discovered, Lake Commandant).

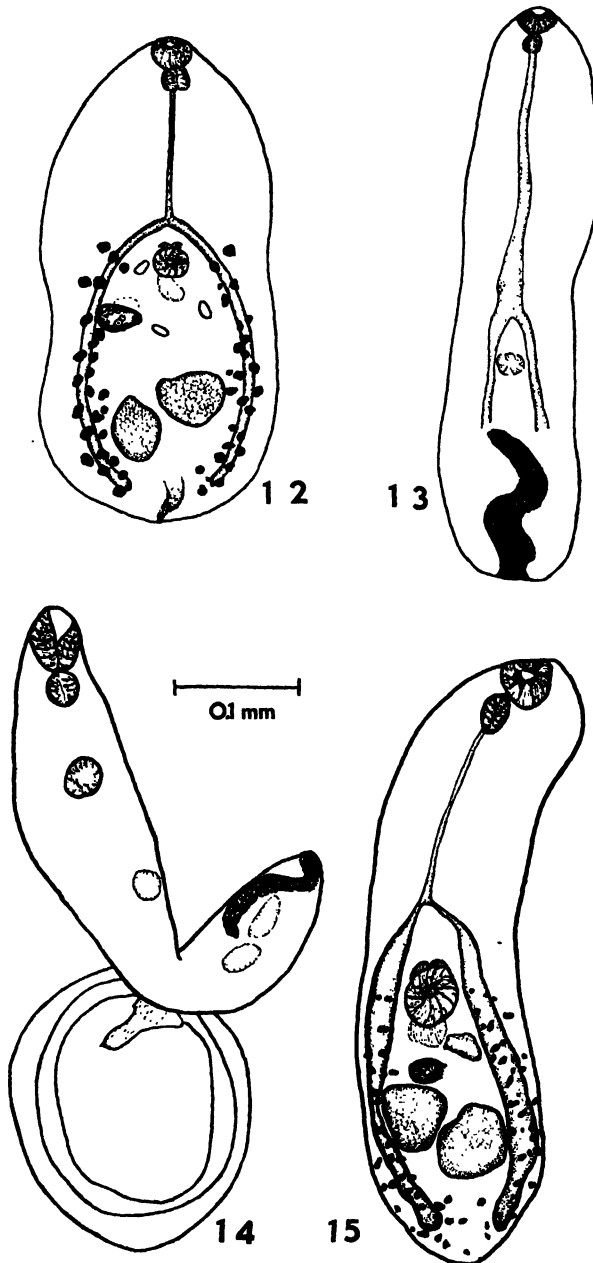
Metacercaria (Fig. 3)

The black pigmented cyst containing the metacercaria of *A. imperator* is easily seen in the trout. As in other members of the group, there is a primary cyst, hyaline and strong, within two outer layers. At least one of these is the host tissue surrounding the organism and is heavily pigmented. The primary cyst, 0.231 by 0.154 mm., is evenly oval in outline. The encysted metacercaria is folded upon itself and is very like the same form in the other heterophyids. The oral sucker, pharynx, traces of the oesophagus, and cuticular spines are distinguishable. The body contains many crystalline objects and others that resemble oil-droplets. The terminal area is filled with a mass of dark globules.

Efforts to cause excystment by artificial digestion failed with a single exception. This specimen probably represents a case of precocious development. All organs were present and there were three or four eggs in the uterus (Fig. 12). Other specimens freed by mechanical means never showed such advanced development. Rudimentary gonads, an S-shaped excretory sac filled with black material, and the faintly outlined acetabulum were usually present (Figs. 13 and 14).

The "black spots" occur on the ventral and lateral parts of the body on the skin just under the scales, and are frequent on the fins, tail, operculum, jaws, inside the mouth, on the gill bars, etc. (Table I). In only two fish, so heavily infected that they were almost uniformly black, were there cysts in the flesh, and even then only a few occurred. There seems to be no selected site for encystment such as Cameron found for *A. venustus* in the catfish.

In addition to Lakes Commandant and St. Bernard, infected trout have been reported from many lakes in the Commandant area. Curiously enough,



FIGS. 12 - 15. *Apophallus imperator* sp. nov. FIG. 12. Precocious excysted metacercaria. FIGS. 13 AND 14. Excysted metacercariae. FIG. 15. Seven-day-old adult.

of neighbouring lakes with apparently very similar characteristics and common outflow, observers have noticed that one will show infected trout and the other will be free. A small portion of trout skin sent by the Federal Department of Fisheries from a New Brunswick lake carried similar cysts. Hunter,

TABLE I
DISTRIBUTION OF HETEROPHYID BLACK SPOT IN TROUT

Body area	No. of cysts per sq. in.		
Base tail fin	54	43	34
Tail fin	61	116	10
Lateral line—dorsal fin	80	37	29
Dorsal fin	47	64	6
Pectoral fin	100	112	22
Base pectoral fin	80	50	21
Gill cover (external)	63	65	12
Gill cover (internal)		5	
Gill arch		4	3
Peduncle		10	7
		(total)	(total)
Anal fin		50	7
Pelvic fin		40	10

These cysts were counted in three specimens selected at random. They were also found in the mouth, tongue, and eyes but were not counted there.

and Hunter and Hunter have twice reported black cysts on trout in New York State. In one instance from Lake Titus they refer to them as Strigeids (13). In the other, from Canopus Creek, they term them only "Black spots" (12). A rainbow trout (*Salmo irideus*) from the collection of the late Mr. Atkinson from the Lake Commandant area shows cysts similar to those of *A. imperator*. Whether these are the same parasite and identical with *A. imperator*, it is impossible to say, but the two are certainly very alike.

A paper presented by Linton (17) at the International Zoological Conference in 1907 is of interest in view of the present information. At that time he reported a black-spotting infection of trout from Alder Lake in the Catskills. The limited information and conditions he gives suggest that the metacercariae he found were not heterophyids. The cysts were larger than those of the present form, and he saw structures in the metacercariae that were probably oral and ventral suckers and holdfast organs. This fact, and the part apparently played by kingfishers, suggest that his material was of strigeid relationship. This supposition is further supported by the presence in Alder Lake of potential snail vectors of this family. One small metacercaria in which he noted only an oral sucker and a pharynx could possibly have been a heterophyid but this cannot be judged with any certainty.

All the speckled trout (*Salvelinus fontinalis*) caught from Lake Commandant were infected. The part other fish might play in maintaining an infection is of interest. Several yellow perch (*Perca flavescens*) and common suckers (*Catostomus commersonii*) (usually found associated with infected trout) failed to show any evidence of heterophyid infections. Similarly lake trout (*Cristivomer naymaycush*) seemed free. These fish were all carefully examined, then subjected to artificial digestion. No cysts were recovered.

The Egg (Fig. 2).

The egg is brownish yellow and oval in shape, usually narrowed at the operculated end and rounded at the other where a small protuberance often occurs. Variations to this general scheme are common. Size is fairly uniform, 0.028 by 0.16 mm. to 0.034 by 0.017 mm. Some are embryonated when voided by the host but all contain miracidia within three weeks. Hatching was not accomplished in the laboratory and mechanical rupture of the egg always destroyed the miracidium.

Experimental Investigations

An effort was made to identify the snail-vector. It has been shown previously that related genera require an operculate snail for development. By far the commonest snails in the infected waters are *Amnicola* sp., but exposure experiments with these snails and hatchery-raised trout have been unproductive. A small variety of *Campeloma* found in the lake was also used in this way with a similar failure. Trout and other fish were exposed to the snails of the non-operculate genus *Helisoma* taken from Lake Commandant. In this case the trout remained uninfected but black pigmented strigeid cysts developed in the other hosts.

In an effort to judge what might be the natural final host of this organism, various laboratory animals were fed cysts or infected fish (Tables II and III). The birds and rodents used had all been raised in the Institute. The cats came partly from the Poultry Department, Macdonald College, and partly

TABLE II
INFECTION TRIALS WITH EXPERIMENTAL ANIMALS

Host	Period of infection	Result of faecal examination	Result of post-mortem examination	Remarks
Cat	7 weeks	Positive	Adult <i>A. imperator</i> in small numbers	Cat fed eviscerated trout
Cat	7 days	—	Immature <i>A. imperator</i>	
Cat	10 weeks	Positive	—	Faecal examinations negative until fourth week and after tenth week
Cat	12 days	—	Adult <i>A. imperator</i> recovered	
Pigeon	7 weeks	Positive fifth week	Adult <i>A. imperator</i> recovered	Fed cysts only
Pigeon	12 weeks	Positive sixth week	Negative	
Duck	12 weeks	—	Negative	—
Duck	8 weeks	May have been positive at six weeks	Negative	A single egg was recovered from this duck
Duck	6 weeks	Negative	Negative	Fed filleted fish: (all the other birds were fed cysts only)
Chick	6 weeks	Negative	—	These chicks were 2 to 3 weeks old when fed cysts
Chick	5 weeks	Negative	Negative	
Heron	8 weeks	—	Negative	
Porcupine	4 weeks	—	Negative	Fed diced cyst-bearing fins

TABLE III
SMALL RODENT INFECTION TRIALS

Rodent	Interval between attempted infection and post mortem	Results			Remarks
		Intestine	Other organs	Faecal examination	
Guinea pig	5 weeks	Negative	Negative	Negative	Fed diced skin and fins, containing many cysts
Guinea pig	11 days	Negative	Negative	Negative	
Mouse	3 weeks	Negative	Negative	Negative	Fed cysts only
Mouse	2½ weeks	Negative	Negative	Negative	Fed diced infected skin
Mouse	2 weeks	Negative	Negative	Negative	Fed diced infected skin
Mouse	5 days	Negative	Negative	Negative	Fed diced infected skin
Mouse	3 days	Negative	Negative	Negative	Fed cysts only
Mouse	12 hours	Negative	Negative	Negative	Fed diced infected skin

from a known source at Kirkdale, Que. In no case had any experimental animals previously eaten fish.

In the experiments the intestine was examined in four sections, duodenum, remainder of small intestine in two equal portions, large intestine. No adult flukes were ever taken from the large intestine or duodenum, and most were found in the latter part of the small intestine.

The results of these attempts to infect various experimental hosts are notably irregular. They do indicate, however, that both mammals and birds may be parasitized by *A. imperator*, and that absence of eggs in the host faeces does not indicate freedom from infection. The appearance of eggs in faeces was considerably later than the earliest recovery of egg-bearing trematodes. The adult state is reached between the seventh and the twelfth days, and eggs first appear in the faeces almost a month later. Egg production was never very great, but no planned egg counts have been taken except to determine when they might be no longer present.

The table shows that the trematodes are not recoverable from the lumen or mucosa of the intestine after an interval. The exact length of this interval and the final fate of the parasites have yet to be determined.

The attempt to produce infections in small rodents was planned as an investigation preliminary to trials on the effects of heat on the encysted metacercariae, to determine the treatment necessary to make infected fish safe for human consumption. The results were uniformly disappointing. Fish scales were sometimes recovered, some of them still carrying the characteristic black pigment, but no cysts or freed trematodes were ever met with in the lumen or tissue of the intestine, or elsewhere in the animals.

The generic distinction recommended by Ciurea (10), based on the time required for development of egg-bearing adults, will not apply to *A. imperator*. He pointed out that members of the genus *Apophallus* were egg-bearing only

by the fourth day, while eggs could be demonstrated in those of the genus *Rossicotrema* by the second day after infection of the final host. Two extremes for egg-development represented by the writer's material are the metacercariae already mentioned, and non-ovigerous specimens taken from a cat on the eighth day after infection (Fig. 15).

In addition to these experimental hosts, the following fish-eating wild birds from the infected areas were examined with negative results, *Ardea herodias*, *Mergus merganser*, *Lophodytes cucullatus*, *Strix varia*. So far, members of the genus reported from Canada are *A. venustus* from various hosts, and *A. brevis* from the loon, *Gavia immer*.

Conclusions

Experimental observations, study of material, or analysis of literature on the genus *Apophallus* and related genera have shown that:

1. *Salvelinus fontinalis* in Quebec is the intermediate host of *Apophallus imperator* sp. nov., a heterophyid trematode.
2. *A. imperator* will become adult in the latter part of the small intestine of birds and mammals, experimentally in pigeons and cats.
3. The primary host is unknown. Snails of the genera *Amnicola*, *Campeloma*, and *Helisoma* from an infected area failed to cause the condition in trout in experimental trials.
4. The arrangement of the vitellaria, body-shape, position of testes, and period of development do not show adequate generic or specific characteristics in *Apophallus* and associated genera.
5. The position and number of gonotyls are of generic significance in the family Heterophyidae.
6. The nature of the gonotyls is of value in specific diagnosis within the genus *Apophallus*.

Acknowledgments

The author offers his very sincere thanks for the interested advice and support of the Director and staff of the Institute of Parasitology, and for the valuable assistance of various individuals and organizations in collecting materials. Mr. Frank Dale, other members and employees of the North Lake Fish and Game Club; Mr. L. J. Jorgensen and his staff at the Seignior Club; Mr. Leslie Bates; members and staff of the St. Bernard Fish and Game Club, particularly Messrs. J. W. Mochon and J. MacMurray; Mr. B. W. Taylor and his assistants of the Hatcheries Branch of the Provincial Service of Game and Fisheries; Mr. Charles Fremont, of the same service, the Federal Department of Fisheries, and others, were generously co-operative. The donation of experimental animals by various individuals was also helpful.

The names of fish are those used by Hubbs (11), birds were identified following Taverner (26), and the names of snails are from the recent check list prepared by LaRocque (15).

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

APRIL, 1940

NUMBER 4

CANADIAN WILTSHIRE BACON

I. OUTLINE OF INVESTIGATION AND METHODS¹

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W. H. WHITE²

Abstract

This paper introduces an investigation involving bacterial, chemical, and physical measurements on bacon and pickle from 22 packing plants. The objects were to determine the over-all variation occurring in practice, the general sources of the variation, and the specific effect of certain curing practices. Methods employed for shipping the samples, sampling, and analyses are described.

It was found that small samples of curing pickle change slightly in nitrite and bacterial content during shipping periods approaching a week's duration, but that these changes are of negligible importance from a practical standpoint if the pickles are kept at temperatures near the freezing point.

Introduction

During recent years the export of bacon as Wiltshire sides* from Canada to Great Britain has assumed considerable importance in Canadian agricultural economy. As a result, investigation of the many problems associated with the manufacture and export of this type of bacon has become increasingly necessary. The present paper, introductory to a series forthcoming from these laboratories, outlines the scope of an investigation into a number of these problems, and describes the methods used for transporting the experimental sides and pickle from the packing plants to the laboratory, and the sampling and analytical methods employed.

The packing plants engaged in the export bacon trade in Canada are widely distributed and the period required for transporting the product naturally varies considerably. A survey of the methods used for making Wiltshire sides showed that, although the individual plants adhered quite closely to their own particular method, there was considerable variability in the practices

¹ Manuscript received January 18, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 34 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 893.

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* For a general description of Wiltshire sides, method of curing, and typical compositions of bacon and pickle, the reader is referred to the publications of the Food Investigation Board, London, England (1-4).

adopted in different plants. This survey, however, gave little indication of the extent to which these variations affected the composition and quality of the bacon. Consequently, the present investigation was undertaken to determine: the over-all variation of the bacon with respect to the several measurements; the main general sources of variation; and more specific information on the effect of certain curing practices.

The over-all variation of Canadian bacon with respect to an individual property may be divided into: differences between sides from the same plant; differences between sides from different plants; and differences due to age from curing. The differences between sides from the same plant represent the combined effect of inherent differences in the curing quality of different carcasses and slight variations in the treatment each carcass may receive from the time of slaughter to the baling of the finished product. The difference between sides from different plants includes the sources of variation recognized above within factories, and also the additional effect of the different practices and pickle compositions used in different establishments.

Although a large number of factors may affect the composition and quality of bacon, particular attention has been given to the effect of the number of "stitches" (injections) per side used in pumping, the number of days in cure, and the composition of the pickles used. This approach to the problem was favoured, as curing is fundamental to bacon production. The particular factors mentioned above are apparently considered to be the most important, since each plant adheres closely to its chosen practice respecting them. Again, if necessary to improve quality, these factors could be modified promptly and inexpensively.

The relation between pickle and product is a function of the scale on which the curing practice is conducted, as shown by the fact that small pieces of pork "cured" in the laboratory (7) may have a salt content more than twice that of the factory product. If the results of investigations into the relations between pickle and product are to be applied in practice, it is essential that the materials studied be typical of those used or produced on a commercial scale. The practices followed in the 22 plants represented in this study varied sufficiently to give considerable information on the effect of pickle composition and other factors on the quality of the bacon.

It was necessary to employ statistical methods to interpret the large body of data obtained, while retaining certain confidential details. For details of the statistical methods used in this and subsequent papers the reader is referred to Snedecor (8).

Method of Shipping Samples

Both pickle and bacon are subject to chemical and bacteriological changes if held at ordinary temperatures, and special arrangements had therefore to be made for transferring these materials relatively unchanged from the plants to the laboratory. The methods employed are described below.

BACON

The period of transport from the plants to the port of Montreal varies from less than one to six days. To minimize abnormal changes during transport, the experimental sides were shipped with regular export shipments in a refrigerator car to the docks at Montreal. Immediately a car was opened, the samples were transferred to a room at 0 to 2° C. When the entire shipment of samples had been accumulated it was transferred to the laboratories by refrigerated truck.

PICKLE

Effect of Age and Temperature on Composition

Before undertaking these studies it was necessary to determine the conditions under which pickle may be shipped for periods of about five days duration without significant change in its chemical composition or bacterial content. In some preliminary experiments tank pickles, taken at the beginning, middle, and end of cure, were stored at 1.1 or 4.5° C. and at 25° C. for periods of about a week, in small sealed jars under aerobic conditions, since air was almost certain to be present in the test samples. Analytical results indicated that the chloride and nitrate content remained constant, while the nitrite content and bacterial numbers showed greater variation from day to day than could be accounted for by experimental error. This led to a more extensive study of the changes in nitrite content and bacterial numbers of pickles obtained from one plant at various stages of cure.

The changes in the nitrite content of five of the above pickles, representing the several types of change observed during storage at 25 and 4.5° C. or lower, are shown in Fig. 1, and the changes in the bacterial content in Fig. 2. The points in these figures represent the means of the determinations made at each sampling, and the ordinate of the cross-hatched section the difference necessary for statistical significance computed from the standard error of the replicated tests.

The nitrite content was determined colorimetrically by a procedure already described (9). The curves on the left-hand side of Fig. 1 were plotted from values obtained by comparing the colour developed in the sample with that of a standard solution of nitrite in a visual colorimeter. Those on the right-hand side were obtained with a photoelectric colorimeter, which yielded more precise results as shown by the smaller necessary difference.

Bacterial numbers were determined on nutrient agar and on 10% salt agar with incubation at 20° C. In Fig. 2 the ordinate represents the changes from the initial value during storage, these changes being expressed as the change in the logarithm of the bacterial number per ml. of pickle. The number of organisms observed on the medium containing 10% salt was much higher than that observed on salt-free media. The actual number present was not of particular interest in this study but will be discussed more fully in later papers of this series.

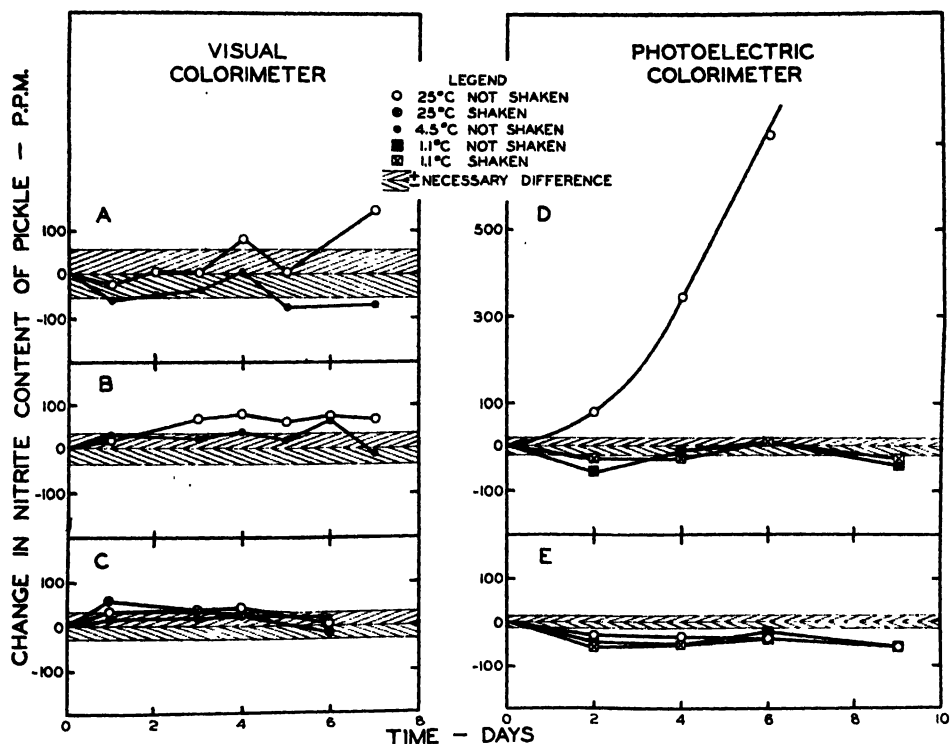


FIG. 1. Changes in nitrite content of pickle during storage at 25° C. and 4.5° C. or lower.

The curves in Fig. 1 show that storage temperature had little effect on the nitrite content of certain pickles (*C* and *E*), while others show a small (*A* and *B*), or decided (*D*), increase in nitrite content during storage at room temperature. Fig. 2 shows that the bacterial number observed on a salt-free medium remains relatively constant during storage at 4.5° C. or lower, but decreases during storage at 25° C. The counts observed on a medium containing 10% salt vary with time, but there is no consistent effect of temperature. These results showed definitely that the changes in nitrite content, and bacterial counts by certain methods, are reduced by keeping the pickle at a temperature of 4.5° C. or lower. Methods for maintaining these low temperatures during transport for periods up to five days will be described in the next section.

The results obtained with pickles *C*, *D*, and *E* (Fig. 1) and *D* and *E* (Fig. 2) indicate that shaking, comparable with that occurring in transport, had no appreciable effect on the nitrite content or bacterial numbers.

It is evident from Figs. 1 and 2 that even when the pickles are kept at temperatures of 4.5° C. or lower, the changes in nitrite content and bacterial numbers during storage may exceed the "necessary difference", or the day-to-day variation attributable to experimental error. The majority of these changes must therefore represent real alterations in composition. These

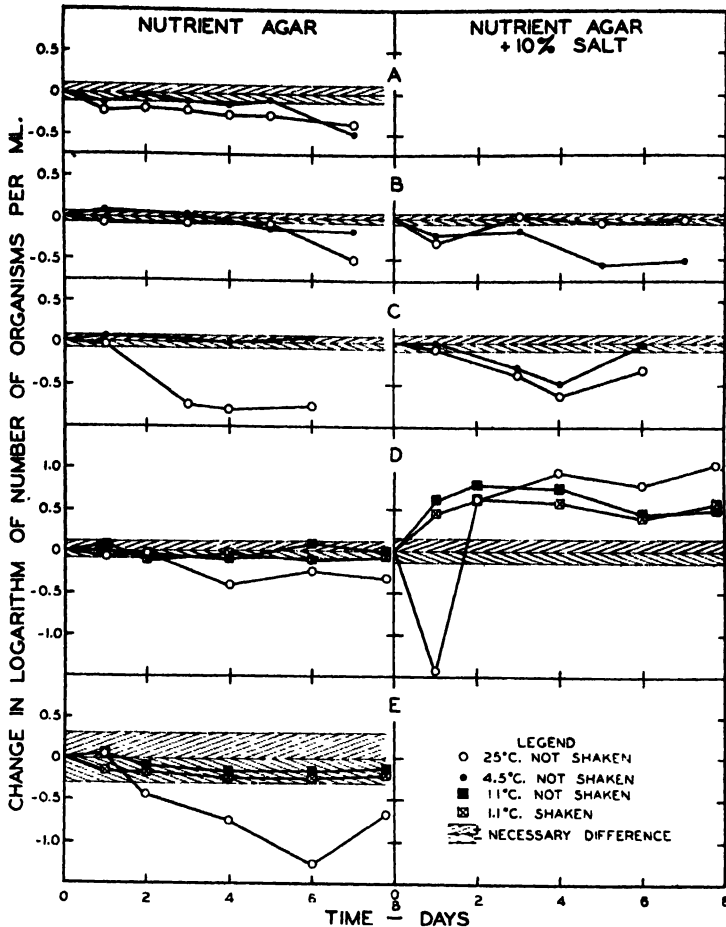


FIG. 2. Changes in bacterial numbers in pickle during storage at 25° C. and 4.5° C. or lower.

changes appear to be of two types: those showing a maximum or minimum during the storage period as shown by pickles A and B in Fig. 1, and B and C (10% salt medium) in Fig. 2; and those showing an increase or decrease only as shown by pickle E in Figs. 1 and 2. The analyses on these and other pickles failed to show any definite relation between the type of change and the stage of curing, i.e., whether the pickle was taken at the beginning, middle, or end of cure. Likewise the changes appear to be relatively independent of the original nitrite or bacterial content. It seems probable that some of the rather sudden changes in composition may be related to such phenomena as protein precipitation. It was therefore concluded that although pickles stored at low temperatures may show small but definite changes in composition, these changes depend on unknown properties of the individual pickle and are consequently unpredictable. Ingram's (5) conclusion that tank pickles may be shipped for periods up to 10 days at temperatures below 10° C.

without appreciable change in their nitrite content does not appear to apply generally.

The differential changes in different pickles during transport must therefore be recognized as an additional source of error, to be combined with the sampling and experimental errors. By obtaining two samples of pickle from each plant at different times, it was possible to compute an over-all estimate of the experimental error, errors due to differential changes during transport, and the error of sampling the tank. The significance of differences between the composition of pickles from different plants could therefore be assessed by comparison with the variance within plants at different times. It is possible that this latter value yields an exaggerated estimate of the error attributable to sampling, changes, etc., since it is almost certain that the composition of the original pickle from an individual plant varies, at least slightly, from time to time.

Although a decrease in the bacterial content of pickle, as indicated by growth on nutrient agar at 20° C., appears to be associated with an increase in nitrite content during storage at 25° C. in these experiments, little significance can be attached to this observation. The small containers used were by no means typical of the storage tanks employed in practice, and the experiments were not conducted on a sufficiently extensive scale to permit definite conclusions.

Containers for Maintaining Low Temperature During Transit

The results of the previous section showed the necessity for using containers that would maintain the pickles in the vicinity of the freezing point during shipment to the laboratories, i.e., for periods up to four or five days' duration.

Four types of containers were tested: (i) a common "thermos" bottle of half-pint capacity; (ii) an insulated commercial isothermal jug of one gallon capacity containing a half-pint jar of pickle and 7 lb. of ice; (iii) a gallon pail insulated with 1½ to 2 in. of cork and containing 4½ lb. of ice in addition to the pickle jar; and (iv) a 2-gal. can insulated with 2 to 2½ in. of wool felt and containing 15 lb. of ice. Typical results given in Table I show that the last container only was satisfactory, and this type was used throughout the investigation. The majority of the experimental samples were received at 0° C., a few at 5° C., and only an occasional one at 10° C. or higher.

Method of Sampling

BACON

The 44 sides of Wiltshire bacon, submitted by the 22 plants, were sampled for analysis three times: (i) on receipt at the laboratories, representative of their condition when shipped from Montreal; (ii) after 10 days' storage at 1.1° C., representative of their condition on arrival in London, England; and (iii) after smoking for 14 hr. at 43 to 46° C., which yielded material approximately representative of that in the British retail store. English practice favours smoking periods of 36 to 48 hr. at relatively low temperatures

TABLE I
TEMPERATURE IN °C. OF PICKLE KEPT IN CONTAINERS OF VARIOUS TYPES

Period of exposure at 25° C., days	"Thermos" bottle 1 pint capacity	Insulated commercial isothermal jug, containing ½ pint pickle + 7 lb. ice	Gallon pail insulated with 1½ - 2 in. cork dust, containing ½ pint pickle + 4½ lb. ice	2-gal. can insulated with 2 in. wool felt, containing ½ - 1 pint pickle + 15 lb. ice
0	2.3	0.2	0.0	0.0
1	12.5	1.1	0.0	0.0
2	17.8	16.7	1.0	0.0
3	21.0	—	11.1	0.0
4	22.5	—	19.7	0.0
5	—	—	—	12.5

compared with Canadian methods. (In these studies the ham was the only portion of the side to be smoked. For this and other reasons the smoking period was reduced.)

On receipt at the laboratory the sides were placed in a room at 4.5° C. and all sampling was done at this temperature. The sides were unwrapped and samples for determining the bacterial number taken first. After removal of material for the chloride, nitrate, nitrite, moisture, pH, colour, colour stability, and tenderness determinations, and measurement of the oxidation-reduction potential of the whole meat in the ham, the sides were rebaled and transferred to a room at 1.1° C. After 10 days' storage the bales were again opened and sampled for these determinations, with the exception of tenderness measurements. This completed the sampling for bacterial numbers, chloride, pH, and oxidation-reduction determinations, but the ham from each side was smoked and again sampled for the other determinations.

Samples were also taken from the sides for studies on salt distribution, the effect of heat treatments comparable with smoking, and the fats. The samples used for these experiments will be described in the papers reporting the results.

The exact position from which the samples were taken and certain details of sampling can best be described by reference to Fig. 3. Samples of the pleural membrane over the second and third, and over the ninth and tenth ribs were removed for making bacterial counts at each sampling. At the first sampling the fourth and fifth ribs were removed without contamination of the pleural membrane and stored separately in an atmosphere of 95% relative humidity at 1.1° C. for a longer period than the sides were stored, to determine the surface bacterial number when visible slime became evident.

Material for the other determinations was obtained after the ham was removed, as indicated in Fig. 3. Samples for chemical analysis and the pH measurements were obtained by cutting a slice from the ham, while a triangular section removed from the side provided material for colour, colour stability,

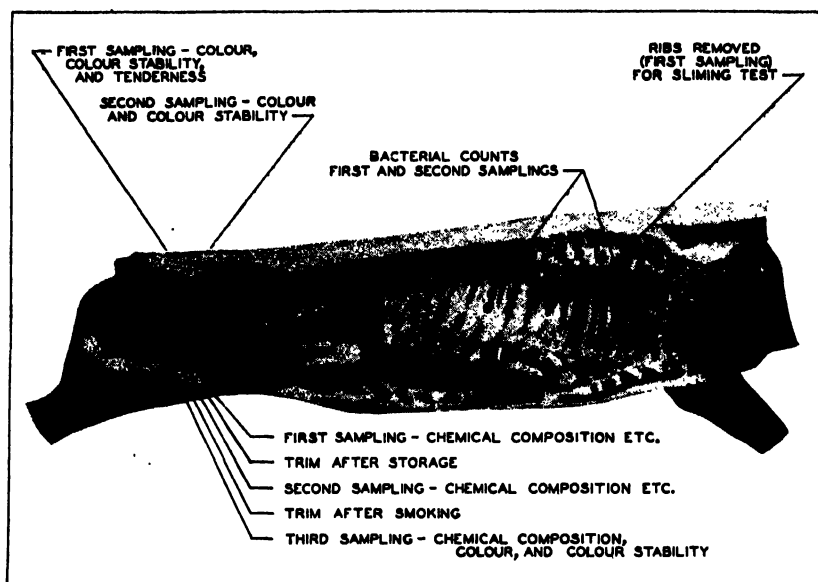


FIG. 3. Portions of Wiltshire side used for determining bacterial counts, chemical composition, and physical properties at each sampling.

and tenderness measurements. Oxidation-reduction potential was obtained by inserting a small pointed platinum electrode into the ham after removal of the slice for chemical analysis.

After storage the cut surface of ham was trimmed and another slice removed for analysis. At the same time a piece was removed from the side for colour and colour stability measurements. Oxidation-reduction potentials were again taken as described above. After smoking the ham was trimmed and another slice removed for both the physical and chemical measurements.

There are certain advantages and disadvantages in this more or less systematic method of sampling. Apart from certain practical limitations, it seemed desirable, since this study was concerned primarily with variations within and among plants, to take the samples from all the sides, at a given sampling, from comparable regions. This should permit more precise comparisons between the compositions of the same portion of different sides than if the samples had been taken at random from the whole side, or a pre-selected part of it. This procedure, however, sacrifices precision in determining the effect of ageing, for although the portions taken from each side at a given sampling are comparable, those taken at different samplings differ systematically in both time and position. In making the statistical analyses it is possible to determine the variance attributable to "between samplings" but it cannot be stated with assurance whether such differences represent a real change with time, or a difference between positions that existed originally. This subject will be discussed further in relation to the results obtained.

The slices of ham taken at each sampling for chemical analysis were immediately trimmed free of fat, bone, and connective tissue. Three small samples representing the outside, centre, and inside of the slice were removed for a study of salt distribution to be described in a later paper. The remainder of the lean meat was ground and thoroughly mixed by passing it through a food chopper several times. The pH of this ground material was immediately determined with a glass electrode, after which it was placed in a moisture-tight sample bottle, frozen in a room at -29°C . and held at this temperature until required for analysis. Storage in the frozen state usually did not exceed a few days. The samples were thawed by placing them in a room at 4.5°C . for a few hours. They were then mixed thoroughly and portions for analysis weighed out in the same cold room. The sample residues were refrozen immediately to preserve the material for any determinations that required to be repeated. Extreme care was necessary at this stage since ground material kept at 4.5°C . for more than a few hours showed a significant increase in nitrite content, and this change was accelerated by exposure to higher temperatures.

All the above measurements were objective, and no attempt was made to estimate subjective qualities such as flavour. A special study of this important attribute of quality has been undertaken.

PICKLE

Two distinct pickles are used for curing Wiltshire bacon. One of these, designated "pump" pickle, is injected into the sides before they are placed in the curing vats, while the other, designated "tank" pickle, is used to cover the sides. Tank pickle suffers progressive changes in composition during the curing period. In order to obtain some estimate of the magnitude of these changes a sample of the tank pickle was taken when the sides were put in to cure, and again when they were removed. These two samples are subsequently designated "cover" and "spent" pickle respectively. When an estimate of the composition of tank pickle was necessary for certain computations, this was taken as the average composition of the cover and spent pickle with respect to the constituent in question.

Corresponding samples of pump, cover, and spent pickles were obtained twice from each plant with an interval of about a month between samplings. The second set of samples was taken from the pickles used for curing the bacon from which the experimental sides were obtained. In view of the small difference observed between successive pickle samplings, the mean composition of the two pickles was used for studying the relations between pickle and product in order to reduce the obscuring effect of experimental errors. The results of certain determinations on the pickles were lost or excluded as a result of errors in sampling, failures in transport, or losses in the laboratory.

When the pickle samples were received they were unpacked and placed in a room at 0°C . until the analyses were complete. Samples for bacterial numbers were removed to sterile glassware, plated, and incubated imme-

diately. Sufficient material for the chloride, nitrate, nitrite, pH, and oxidation-reduction potential measurements was removed to an ordinary laboratory for analysis and these determinations were made at room temperature. As the proteins tend to precipitate from pickle at room temperature the samples for this determination were pipetted from the original sample at 0° C. The remainder of the sample was filtered and colour measurements made in a room at 4.5° C.

Methods of Analysis

The methods employed for making the bacterial counts on both bacon and pickle will be described in later papers reporting the results of the measurements. The procedures followed in determining chloride, nitrate, and nitrite have already been described (9).

The protein nitrogen content of the pickle was determined on suitable portions (usually 10 or 25 ml. depending on the protein nitrogen content) pipetted into Kjeldahl flasks in the cold room. Nitrate nitrogen was removed (6, p. 27) before the Kjeldahl digestion.

The moisture content of the bacon was determined on 2- to 3-gm. portions of the ground material, by drying in flat aluminium dishes to constant weight (16 to 24 hr.) at 100° C. Preliminary measurements at lower temperatures yielded the same results, within experimental error, on this relatively fat-free material, but a much longer drying period was required. Actually it was difficult to attain a truly constant weight at any temperature, but after drying for 16 to 24 hr. at 100° C. the loss of weight over an additional 4-hr. drying period was never more than the equivalent of 0.05% moisture. Differences of this magnitude were smaller than the error between duplicates, and considerably less than the over-all sampling error.

The pH and oxidation-reduction potential measurements were made with a Bechmann pH meter. The appropriate standard electrodes supplied with the instrument were used for the determinations on pickles. The pH measurements on bacon were made in a room at 4.5° C., using a large glass electrode and extension leads. Both the glass and calomel electrodes were forced into a portion of the ground sample. Readings were required to check within 0.05 pH at different positions in the sample. Standard buffer solutions were used for adjusting the instrument at this low temperature, and all reported values were corrected to 20° C.

The oxidation-reduction potential of the bacon was measured with a special platinum electrode consisting of a 60° platinum cone, $\frac{1}{4}$ -in. diameter at the base, carried on a $\frac{1}{4}$ -in. diameter bakelite tube. This electrode was inserted to a depth of 2 to 2½ in. into the ham and the circuit completed by inserting the calomel electrode into a cut at the surface. The observed potentials were converted to Eh by correcting for the potential of the calomel electrode at 4.5° C. The instrument and electrodes used for the Eh measurements were checked with a buffer solution of known pH containing quinhydrone. Several difficulties were encountered in making the Eh measurements in both bacon

and pickle, and there was some uncertainty in the results obtained. These will be discussed more fully when the results are presented in a later paper.

The colour and tenderness measurements on the bacon were made by means of instruments and methods already described (10, 11). These observations were made on slices or portions of whole meat, immediately after cutting, in a room at 4.5° C. Samples were chosen that were free from obvious streaks of fat or connective tissue. An estimate of colour stability was obtained by repeating the measurements on these slices periodically during exposure to air at a temperature of 10° C. and a relative humidity of 95%. This condition avoided serious drying of the samples, which has been shown to affect the colour (12). The results therefore indicate the stability of the colour to atmospheric oxidation at a temperature commonly prevailing in the storage chamber of a retail store.

Acknowledgments

The authors wish to express their appreciation and thanks to the management and staffs of the packing companies for their cordial co-operation in providing certain confidential details relative to their curing practice, for donating the bacon and pickle for analysis, and for collecting and forwarding these samples. These include: Burns and Co. Ltd., at Calgary, Edmonton, and Prince Albert; Canada Packers Ltd., at Toronto, Peterborough, Hull, Montreal, and Edmonton; Dumarts Ltd.; F. W. Fearman Co. Ltd.; First Co-Operative Packers of Ontario; Fowler's Canadian Co.; Gainers Ltd.; J. M. Schneider Ltd.; Swift Canadian Co. Ltd., at Toronto, Winnipeg, Moose Jaw, and Edmonton; Wellington Packers Ltd.; Whyte Packing Co.; Wight and Co. Ltd.; and Wilsils Ltd. In addition the following firms furnished pickle and all other information requested but were unable to supply bacon at the time this phase was investigated: Burns and Co. Ltd., at Regina and Winnipeg; Canada Packers Ltd., Winnipeg; and Union Packing Co., Calgary. Special thanks are tendered to the management and staff of Canada Packers Ltd., Montreal, for assisting in the collection at Montreal of the bacon from all plants and its transport to Ottawa, and of Canada Packers Ltd., Hull, for smoking the test material and assisting with the sampling. It would have been impossible to conduct the investigation without the whole-hearted co-operation of these firms.

Thanks are also tendered to the officers of the Dominion Department of Agriculture, particularly those of the Marketing Services, for assistance in making the general arrangements, and to that Department for defraying the cost of shipping the samples.

Finally the authors wish to thank Messrs. A. E. Chadderton, A. Morrison, E. A. Rooke, and G. N. Seed, laboratory assistants, National Research Laboratories, for their help at all stages of the investigation, and in particular for their excellent co-operation in promoting the work during critical periods.

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CANADIAN WILTSHIRE BACON

II. CHLORIDE, NITRATE, AND NITRITE CONTENT OF BACON AND PICKLE¹

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Abstract

Analysis of Wiltshire bacon produced in Canadian plants showed that different sides varied in their chloride, nitrate, and nitrite contents, the chloride content being the most uniform. Although the observed variations were statistically significant they do not necessarily affect the quality of the product. An analysis of variance showed that differences between the sides from different plants were the main general source of variation in the chloride and nitrate contents, while the differences between sides from the same plant were the main source of variation in nitrite content. This, and other evidence, indicates that the properties of the individual side affect its nitrite content to a considerable extent.

The variation in the composition of the pickles used in different plants was significantly greater than the variations within plants, although the chloride content was relatively uniform throughout. Other curing practices, such as the number of injections used for pumping a side and the time in cure, also varied between different plants. Statistical computations showed that the number of injections used for pumping was correlated with the chloride and nitrite content of the sides, while their nitrate content was correlated with the nitrate content of the pump pickle. Although these factors affected the composition of the product with respect to each constituent, the level of the correlation coefficients was rather low. It is therefore concluded that most of the observed variation in the bacon was contributed by other unmeasured factors, or by inherent differences between the carcasses.

The analysis of variance showed significant differences between the content of the three constituents at different samplings. The method of sampling, however, did not permit the true effect of ageing to be distinguished precisely from the effect of systematic differences in position, and the observed differences between samplings might possibly have been due entirely to the effect of position.

Introduction

Although the quality of bacon may be affected by many factors, relatively few of these can be closely controlled. Those that can be standardized and reproduced from time to time in a given plant include the composition of the pickles with respect to the several salts used, and certain of the curing processes. Different plants, however, use different pickle formulas and curing practices, and this, together with the fact that the composition of the product may depend on other factors that are not closely controlled, can give rise to certain variations in the product. This paper reports the chloride, nitrate, and nitrite contents of the bacon, the general sources of variation, and more specifically, the effect of pickle composition and certain curing practices.

¹ Manuscript received January 18, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 35 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 894.

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An outline of this investigation and a description of the methods used have been reported in the first paper (5) of this series. Statistical methods (7) were employed to reduce the large body of results, and permit their interpretation in terms of the required information.

The general mean and the standard deviation of a single observation served to describe the composition and over-all variation of the bacon and pickle with respect to each of the constituents. The coefficient of variability was computed from these quantities to facilitate comparison of the relative variability of different constituents. The practical significance of the observed variations had naturally to be assessed from other considerations.

The principal sources of variation were determined by an analysis of variance. For bacon, the total variance could be subdivided into portions attributable to: average differences between sides from different plants; differences between sides within plants; average differences between samplings; and sampling and analytical error. Although all the analyses of the samples were made in duplicate, it was impossible, within the limits of available time and material, to obtain and analyse two independent pieces from each side at each sampling. In consequence the residual variance was used to estimate the combined sampling and analytical error.

The results obtained with pickle were analysed in the same way except that only two sources of variance could be recognized, namely, within and between plants. For reasons already given (5) the variance within plants had to be taken as an estimate of the sampling and other errors. This variance probably overestimates these errors, since the samples for each plant were obtained at different times, and consequently the observed differences include any real change in the composition of the pickle from time to time.

The specific effect of pickle composition, number of stitches per side used in pumping, and the number of days in cure, on the concentration of salts in the product, was determined by computing simple correlation coefficients between the quantity of a given constituent in the bacon, the number of stitches per side, the number of days in cure, and the quantity of the same constituent in the pump and tank pickles. In some instances it was possible to combine two of these quantities. For instance the amount of salt contributed by the pump pickle may reasonably be taken as the product of its salt content and the number of stitches used per side. This assumption appears to be valid, since results by Callow (3, pp. 65-70) indicate that the amount of pickle retained is proportional to the number of stitches. Nevertheless some uncertainty remains, since the pumping equipment and method in different plants may not inject the same amount of pickle per stitch, while the number of stitches in the ham portion analysed may not remain in a fixed proportion of the number per side. Similarly the product of the chloride content of the tank pickle and the number of days in cure were used for computing certain correlations.

Finally the composition of the bacon will be affected by the composition and method of application of both the pump and tank pickles. The relative

effect of these two phases of curing cannot be established directly from simple correlation coefficients, since certain factors in each phase may be associated. Thus a plant using a weak pump pickle may also use a weak tank pickle, or a plant accustomed to the production of a mild bacon may reduce both the number of stitches used in pumping and the number of days in cure. By computing partial correlation coefficients, however, it was possible to determine the effect of the pumping practices independent of tank curing practices and vice versa. These computations were made wherever the simple correlations were significant, in order to establish the observed relation independent of associated factors.

Chloride

The mean chloride content of the bacon and pickles from all plants, over all samplings, appears in Table I. The average chloride content of 3.9% and a maximum of 5.8% indicate that Canadian Wiltshire is milder, on the average, than British bacon, if published results (1, 2) are typical of British curing practice.

The chloride content of the pickles is expressed on a weight-volume percentage basis, i.e., grams of sodium chloride per 100 ml. of pickle, or approximately lb. per 10 gal. The figures in Table I show that the mean chloride content of pump pickle approaches saturation (about 31.7% on a weight-volume basis at cellar temperature). Tank pickle is somewhat less concen-

TABLE I
CHLORIDE CONTENT OF BACON AND PICKLE
(As sodium chloride)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	3.93	29.20	28.32	23.80
Standard deviation, %	1.05	1.71	2.67	1.93
Coefficient of variability	26.79	5.86	9.41	8.10

Analysis of Variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.206	19	0.795	16	0.824	16	1.280
Between samplings	1	21.315**						
Between sides within plants	22	0.476*						
Between plants	21	2.652**	(18)	5.178**	15	13.815**	15	6.323**

* Indicates 5% level of significance, in this and subsequent tables.

** Indicates 1% level of significance, in this and subsequent tables.

trated initially and the chloride content naturally decreases during cure, cf., values for cover and spent pickles respectively. Although the greatest absolute variations in chloride content occur in cover pickle, the chloride content of the bacon is relatively more variable than that of the pickles.

The results of analyses of variance are given in the lower part of Table I. Those for bacon show that, on the average, both the variance between samplings from the same side and that between different sides from the same plant were significantly greater than the sampling and analytical errors. The variance between sides from different plants, however, was significantly greater than that between sides within plants. The significance of the variance between samplings will be discussed more fully in a later section. Although it must be recognized that sides cured at the same time in the same tank differ significantly in chloride content, it is obvious that the major portion of the variation is contributed by the different chloride contents of bacon from different plants.

The simple and partial correlation coefficients between the chloride content of the bacon and the chloride contents of the pump and tank (mean of cover and spent) pickles, the number of stitches per side used in pumping, and the number of days in cure, appear in Table II. The simple correlation coefficient between the chloride content of the bacon and the number of stitches used per side is the only one that exceeds the 5% level of significance. In fact, the coefficients between the chloride contents of the bacon and that of both pickles, although insignificant, are negative. This indicates that the chloride content of the bacon is not affected by the observed variations in the chloride content of the pickles used, but depends entirely on other factors.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE CONTENT OF PICKLE AND CHLORIDE CONTENT OF BACON

(As sodium chloride)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean chloride content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.45*
Days in cure	20	-0.33
Chloride in pump pickle (mean)	19	-0.36
Chloride in tank pickle (mean)	19	-0.10
Loss of chloride from tank pickle during cure (mean)	19	-0.33
Chloride in pump pickle (mean) \times stitches per side	19	0.40
Chloride in tank pickle (mean) \times days in cure	19	0.25
Chloride in pump pickle (mean) \times stitches per side, independent of chloride in tank pickle (mean) \times days in cure	18	0.41
Chloride in tank pickle (mean) \times days in cure, independent of chloride in pump pickle (mean) \times stitches per side	18	0.28

The simple and partial coefficients between the chloride in the product and such combined quantities as chloride in pump pickle times the number of stitches, and chloride in tank pickle times days in cure, were insignificant.

Although these results indicate that the number of stitches used for pumping a side is more important than any of the other factors studied, the level of the correlation coefficient, although significant, is so low that less than 20% of the observed variance in the chloride content of the bacon can be accounted for by variations in the number of stitches used. In consequence the major portion of the observed variation in chloride content, including that between sides from the same plant, must be due to unmeasured quantities concerned with curing, or to inherent differences between carcasses. Fortunately the over-all variation observed cannot be regarded as having a serious direct effect on the quality of the product, although it may be desirable to produce bacon of more uniform chloride content.

Nitrate

The mean nitrate content of the bacon and pickles, the standard deviation from these means, the coefficients of variability, and the results of an analysis of variance are given in Table III. The nitrate contents of the pickles are, on the average, comparable with those used in British practice (3, pp. 65-70) and would appear to be satisfactory. The nitrate content of both the product and the pickles is much more variable than their chloride content, as shown by the coefficient of variability. The relatively greater variation observed

TABLE III
NITRATE CONTENT OF BACON AND PICKLE
(As sodium nitrate)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	0.184	2.32	1.32	0.92
Standard deviation, %	0.220	2.469	0.923	0.644
Coefficient of variability	119	107	69.7	70.4

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	0.0125	19	0.342	16	0.380	16	0.056
Between samplings	2	0.0534*						
Between sides within plants	22	0.0228						
Between plants	21	0.2212**	18	12.173**	15	1.354**	15	0.798**

in the nitrate content of the bacon and pump pickle, as compared with that of the cover and spent pickles, was contributed largely by one plant that used much more nitrate than the average in its pump pickle. This practice had a direct effect on the nitrate content of the product.

The analysis of variance shows that there was a significant difference in the nitrate content between samplings. This phase will be discussed later. The difference between sides from the same plant did not exceed the analytical and sampling errors significantly. The difference between both the bacons and pickles from different plants, however, was significantly greater than the observed differences within plants.

The simple and partial correlation coefficients between the nitrate content of the bacon, and the known factors in the curing practice appear in Table IV. These correlation coefficients were computed on two bases, namely, including and excluding the results obtained from the plant using high nitrate concentrations in its pump pickle. By including the results from this plant, the correlation coefficients are based on the figures from which the results in Table III were computed. By excluding the results from this plant it was felt that the correlation coefficients were more typical of the effect of the ordinary variations in nitrate content of the pickles. As can be seen from the table, the exclusion of these results tends to reduce the degree of correlation between the nitrate content of the meat and quantities including the composition of the pump pickle, and to increase that with quantities involving the composition of the tank pickle.

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN NITRATE CONTENT OF PICKLE AND NITRATE CONTENT OF BACON

(As sodium nitrate)

Quantities correlated	Simple correlation coefficients			
	All plants		Exclusion of one exceptional plant	
	D.f.	r	D.f.	r
Mean nitrate content of bacon over all sides and samplings with:				
Pump stitches per side	20	0.13	—	—
Days in cure	20	0.29	—	—
Nitrate in pump pickle (mean)	19	0.92**	18	0.48*
Nitrate in tank pickle (mean)	19	0.59**	18	0.62**
Loss of nitrate from tank pickle during cure (mean)	19	0.09	—	—
Nitrate in pump pickle (mean) independent of tank pickle (mean)	18	0.88**	17	0.29
Nitrate in tank pickle (mean) independent of pump pickle (mean)	18	0.11	17	0.51*
Nitrate in pump pickle (mean) × stitches per side	19	0.89**	18	0.63**
Nitrate in tank pickle (mean) × days in cure	19	0.55**	18	0.57**
Nitrate in pump pickle (mean) × stitches per side, independent of nitrate in tank pickle (mean) × days in cure	18	0.80**	17	0.48*
Nitrate in tank pickle (mean) × days in cure independent of nitrate in pump pickle (mean) × stitches per side	18	0.02	17	0.39

Considering the coefficients given in the last column of Table IV it is evident that the nitrate contents of both the pump and tank pickles affect the nitrate content of the sides. This was to be expected, since the variation in the nitrate content of the two pickles was proportionately far greater than the methods used for their application. The partial correlation coefficients between the nitrate content of the bacon and that of the pump pickle, independent of tank pickle and vice versa, indicate that the nitrate content of the tank pickle has a greater influence on the composition of the product than that of the pump pickle. Nevertheless, the combined quantities, representing the known values of the pumping and tank-curing practices respectively, both yielded simple correlation coefficients that were highly significant. This suggests that the number of stitches per side and the number of days in cure, as well as the nitrate content of the two pickles, have some effect on the nitrate content of the sides. Partial correlation studies indicate that the composition of the pump pickle and the stitches per side are more influential than the composition of the tank pickle and the number of days in cure.

It is concluded from these results that the nitrate content of the pump and tank pickles and the number of stitches used in pumping are the principal factors affecting the nitrate content of the sides. It seems probable that, if more uniform pickle compositions and pumping practices were used in all plants, the relative variability of the product with respect to nitrate would be reduced to about the same level as that reported for chloride (Table I).

Nitrite

The mean nitrite content of the bacon and pickles, the variation of each, and the main sources of this variation are shown in Table V. The bacon con-

TABLE V
NITRITE CONTENT OF BACON AND PICKLE
(As sodium nitrite)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, p.p.m.	26.3	303	536	482
Standard deviation, p.p.m.	24.1	366	376	293
Coefficient of variability	91.6	121	70.2	60.7

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	127.8	19	8,331	16	6,551	16	3,966
Between samplings	2	818.6**						
Between sides within plants	22	1,213**						
Between plants	21	1,743	18	266,669**	15	285,593**	15	172,802**

tained 26 p.p.m. of sodium nitrite on the average. This is well within the 200 p.p.m. of sodium nitrite permitted by the Canadian pure food regulations (6). Although the coefficient of variability shows, on the average, considerable variation in the nitrite content of different sides, none of the sides, at any of the samplings, had a nitrite content approaching the legal limits.

The composition of the pickles with respect to nitrite content appears to be quite typical (3, p. 65-70), although these pickles were analysed during the summer months when the bacterial activity, and consequently the nitrite contents, would probably be maximal. On the whole the nitrite content shows about the same relative variability as the nitrate content.

The analysis of variance for bacon indicates that the difference between samplings and between sides from the same plant is responsible for most of the variance. In spite of the highly significant variations in the nitrite content of the pickles used in the different plants, the variance between the nitrite content of the bacon from different plants was not significant. This result is partly accounted for by the relatively large variance between sides within plants, most of which was contributed by the sides received from three or four plants. Detailed inquiry and examination of the results showed no valid reason for excluding these sides. The present investigation therefore indicates that the difference between sides treated in the same way is the main source of variation in nitrite content. More extensive analyses now under way, however, indicate that there is a significant difference between the nitrite content of sides from different Canadian plants after smoking in England. This cannot be taken as contradicting the present findings, since it will be shown later that the conditions used for smoking bacon in England may result in a differential development of nitrite in different sides.

The correlation coefficients between the nitrite content of the bacon and the known curing practices appear in Table VI. The correlation between the nitrite content of meat and the number of stitches used for pumping a side is the only one that is significant. Since correlations between the nitrite content of the bacon and that of the pump pickle never approach the level of significance, it appears that the number of stitches used for pumping increases the nitrite content of the bacon indirectly, rather than by a direct contribution of nitrite to the sides. This hypothesis is supported by the highly significant partial correlation between the number of stitches per side independent of nitrite in pump pickle. The possible nature of such an indirect effect is obscure, but it may result from the introduction of air or bacteria into the meat during pumping. It will be shown in a later paper that the pump pickles contained a considerable number of aerobic bacteria.

If nitrites can be produced from nitrates either on or within the sides after curing, it is evident that the observed nitrite content of the sides reflects both the extent or rate of this reaction as well as that between nitrite and haemoglobin, or muscle proteins. An attempt was therefore made to determine which of the two reactions was the more important in determining the nitrite content of the bacon. This was done by computing the nitrate : nitrite

TABLE VI

COEFFICIENTS OF CORRELATION BETWEEN NITRITE CONTENT OF PICKLE AND NITRITE CONTENT OF BACON

(As sodium nitrite)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean nitrite content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.57**
Days in cure	20	0.29
Nitrite in pump pickle (mean)	19	0.25
Nitrite in tank pickle (mean)	19	0.25
Loss of nitrite from tank pickle during cure (mean)	19	0.21
Nitrite in pump pickle (mean) independent of stitches per side	18	0.21
Pump stitches per side independent of nitrite in pump pickle (mean)	18	0.57**
Nitrite in pump pickle (mean) independent of nitrite in tank pickle (mean)	18	0.15
Nitrite in tank pickle (mean) independent of nitrite in pump pickle (mean)	18	0.15
Nitrite in pump pickle (mean) \times stitches per side	19	0.34
Nitrite in tank pickle (mean) \times days in cure	19	0.23
Nitrite in pump pickle (mean) \times stitches per side, independent of nitrite in tank pickle (mean) \times days in cure	18	0.27
Nitrite in tank pickle (mean) \times days in cure, independent of nitrite in pump pickle (mean) \times stitches per side	18	0.12

ratio in the pickles and bacon from the individual plants. It was felt that if this ratio was significantly lower in the bacon than in the pickle, production of nitrite in the sides would be indicated, while a higher ratio in the bacon would suggest that reactions favouring the disappearance of nitrite predominated. Moreover, the use of the ratio would tend to minimize the effect of systematic variations in the composition of the meat between the different positions.

These ratios were found to be extremely variable both in the bacon and in the pickle. When they were subjected to an analysis of variance, neither the difference between samplings, between sides from the same plant, or between sides from different plants were significantly greater than the residual variance attributable to sampling and analytical error. The nitrate:nitrite ratio in the tank pickle only showed significant differences among plants. In fact, the mean ratio for the bacon over all samplings and sides did not differ significantly from that of the pickle over all samplings and plants.

It was observed, however, that two sides from each of two plants had a very low nitrite content at the first sampling, and since the nitrate content was about average, the nitrate:nitrite ratio was exceedingly large. The nitrite content at the later samplings, however, had increased considerably, indicating the production of nitrite within these sides. Since the different

sides in this group showed considerable variation in nitrite content at all samplings it was necessary to test the significance of the observed differences. For doing this the nitrate : nitrite ratio was used in preference to the nitrite content, for reasons already given.

Since the mean nitrate : nitrite ratio of these four sides varied widely both between sides and samplings, with the variance approximately proportional to the mean, it was necessary to use the logarithm of these ratios in making the analysis of variance in order to provide valid tests (4) of significance. The results of this analysis appear in Table VII, from which it is evident that the differences between sides and samplings are both significantly greater than the residual variance.

TABLE VII

ANALYSIS OF VARIANCE OF SAMPLES HAVING LARGE
NITRATE : NITRITE RATIOS INITIALLY

Figures on basis of logarithms of ratios

Variance attributable to	Degrees freedom	Mean square
Between sides within times	3	2.01*
Between times (samplings)	2	2.62*
Residual	6	0.412

It is concluded from these results that sides having a high nitrate : nitrite ratio initially, i.e., low nitrite content, may increase in nitrite content during maturation and smoking. Furthermore, the difference between sides indicates that the extent of this increase depends on the properties of the individual sides. Whether the variable increases in the nitrite content of different sides were due to varying bacterial loads or to inherent differences in the carcasses themselves is not known. Since this behaviour was not observed over all samples it appears that under average conditions nitrite production does not occur to any significant extent in samples containing an average nitrite content initially.

Difference Between Samplings

Certain changes, termed maturation, occur in bacon after removal from cure. Although these are believed to be beneficial to the general quality and flavour of the product (3, pp. 70-72), their nature is obscure. It therefore seemed desirable to consider the changes that occurred in all of the individual constituents and properties measured. The results given in previous tables showed that the three constituents dealt with in this paper did differ significantly between samplings. However, as pointed out in the first paper (5), the difference between samplings includes the effect of systematic differences between the positions from which successive samples were taken as well as the true effect of ageing and smoking. Since certain constituents, such as chlorides, should not suffer any change with time, these can be used as reference substances for assessing the significance of observed differences in other constituents which might change, e.g., nitrites.

Table VIII shows the mean chloride, nitrate, and nitrite contents over all sides by samplings, the difference between these means, and the significance of the differences compared with the sampling and analytical error. All three constituents showed a significant increase between the first and second samplings. The chloride content was not determined at the third sampling. Between the second and third samplings, the nitrate content remained practically constant, and the nitrite content decreased significantly. The increased chloride content must represent the effect of position or chloride distribution, which will be discussed in a later paper. A similar conclusion must be reached for nitrate since there is no evidence that any appreciable quantity was converted to nitrite. Since the increase in nitrite between the first and second samplings is of the same order on a percentage basis as that observed with chloride and nitrate, it appears that this increase represents the effect of position rather than time. The decrease in nitrite between the second and third sampling may indicate a decrease in nitrite during smoking, since the nitrate content remains practically constant.

TABLE VIII
DIFFERENCE BETWEEN SAMPLINGS

Constituent	Mean by samplings			Difference between means		
	First (1)	Second (2)	Third (3)	1—2	2—3	1—3
Sodium chloride, %	3.43	4.42	—	0.985**	—	—
Sodium nitrate, %	0.144	0.204	0.205	0.060*	0.001	0.061*
Sodium nitrite, p.p.m.	22.6	31.1	25.2	8.42**	5.83*	2.59

A further attempt was made to determine the effect of age on nitrite content by computing simple correlation coefficients between the nitrite content and the age of the bacon at the three samplings independently, and over all analyses irregardless of samplings. These were found to be -0.04 , -0.20 , and -0.28 , for the first, second, and third samplings respectively, and $+0.02$ over all samplings. None of these coefficients is statistically significant, although the negative sign within samplings indicates a decrease in nitrite with time. When the computation is made over all samplings, the effect of position intervenes, decreasing the correlation coefficient and changing its sign.

In conclusion there is no evidence to indicate that, on the average, a serious change occurs in the nitrite content of bacon during storage at 1.1°C ., or smoking for 14 hr. at about 45°C . There is some indication that nitrite may be formed in sides having a low nitrite level on removal from cure. The difference between the nitrite contents of sides cured in the same plant suggests that much of the observed variability is due to some factor that is not closely controlled in commercial practice. This factor may be some inherent property of the individual sides, or differential bacterial contents.

Difference Between Bacon and Pickle from Different Plants

The results reported in earlier tables show that although the differences between sides from the same plant were generally significant, the differences between sides from different plants were usually greater. It is therefore of interest to determine how the concentrations of the several salts in the sides and pickles from the various plants are distributed around the general mean for each constituent.

Before preparing these frequency distributions, the difference necessary for statistical significance was computed for the variance between sides within plants for bacon, and from the variance within plants at different times for the pickles. These necessary differences were then used as the class interval in preparing the frequency distribution for each constituent. This method has the advantage of distributing the observed values over the number of classes that can be distinguished experimentally from one another, although individual results in adjacent classes may not differ significantly. It must be recognized, however, that a large number of classes will be distinguished if the material is variable with respect to the measurement in question, or if the variance between sides and pickles from a given plant is small, and vice versa.

The frequency distributions for the chloride, nitrate, and nitrite contents of the bacon and pickle appear in Fig. 1. Four classes can be distinguished in the bacon with respect to chloride content. Since the distribution around the mean is symmetrical, with only a few plants falling in the extremes, the

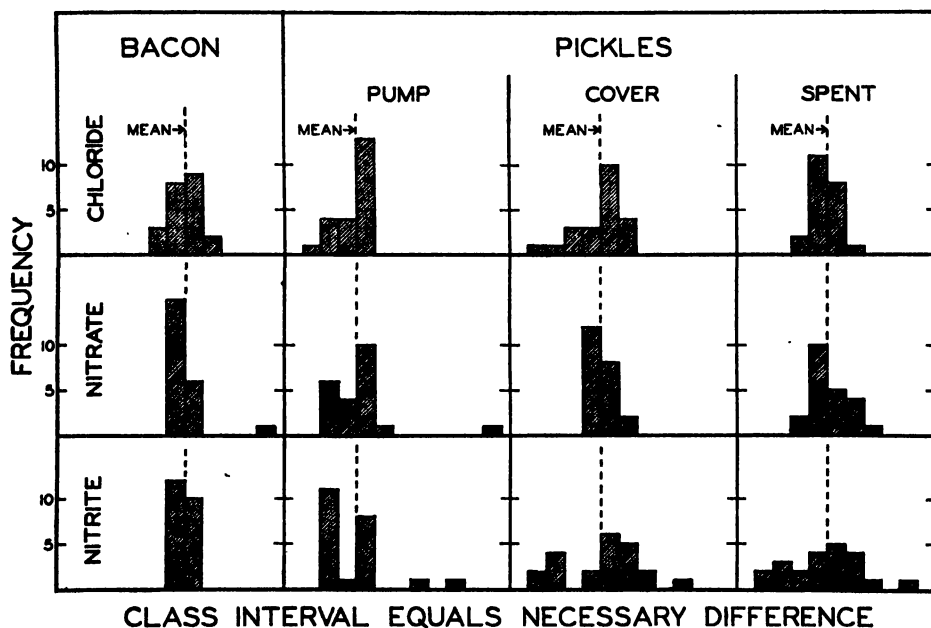


FIG. 1. Frequency distributions of chloride, nitrate, and nitrite contents of bacon and pickle from different plants.

chloride content of the product can be considered satisfactory. With the exception of the one plant using a high nitrate content in its pump pickle, only two classes can be distinguished with respect to the nitrate and nitrite contents of the bacon. This is due to the considerable difference observed between the content of these constituents in sides from the same plant.

The chloride content of the pump pickles falls into four classes, the distribution showing that the majority of the pickles are approximately saturated. Since the necessary difference for the pump and cover pickles was approximately the same, the more variable chloride content of the cover pickle is shown by a distribution over six classes. Only four classes of spent pickle could be distinguished.

The nitrate contents of the several pickles fall into three or five classes. Since the necessary differences were relatively large for this constituent, considerable variability is indicated. It has already been shown (Table IV) that the nitrate content of the pickles affects that of the bacon. It appears therefore that the use of pickles of more uniform nitrate content in the different plants would be desirable.

The nitrite content of the pickles was more variable than either the chloride or nitrate contents, since, in spite of the relatively large necessary differences, the values are scattered over about seven distinct classes. Although no direct relation could be demonstrated between the nitrite content of the pickle and bacon (Table VI), and in fact, there was some indication that the properties of the individual side determine its nitrite content, it would nevertheless seem desirable to standardize the nitrite content of the pickles used in the different plants as far as possible. In this connection it must be kept in mind that the combination of nitrite with the muscle pigments and proteins may occur differentially in different sides, and thus contribute to the observed variability.

In conclusion it should be pointed out that, although these results show statistically significant differences between the chloride, nitrate, and nitrite contents of bacon and pickle from different Canadian packing plants, it is not known that these variations seriously affect the final quality of the product.

Acknowledgments

The authors wish to express their appreciation of the able assistance and co-operation of the individuals and firms referred to in the first paper of this series. Special thanks are due to A. E. Chadderton, laboratory assistant, National Research Laboratories, who was responsible for certain phases of sampling, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for his advice and assistance in connection with the statistical treatment of the results.

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CANADIAN WILTSHIRE BACON

III. pH, OXIDATION-REDUCTION POTENTIAL, AND MISCELLANEOUS MEASUREMENTS ON BACON AND PICKLE¹

BY W. H. COOK² AND A. E. CHADDERTON³

Abstract

The pH of bacon was relatively uniform, but it was possible to demonstrate statistically significant differences between sides cured in the same plants. Sides from different plants were however no more variable than those from the same plant. The results indicate that the pH of the bacon is affected by the pH of the pump pickle, decreases with the time in cure, and increases with the age from cure.

The absolute values of the Eh potentials observed in bacon were doubtful, but since the measurements indicated a statistically significant difference between sides from different plants it appears that this property may be a function of curing practice. Although the moisture content of bacon was relatively uniform, there was a significant difference between sides from different plants, and a significant loss of moisture during maturation and smoking.

The protein content of the tank pickle from different plants varied considerably, and probably reflects the effect of different handling practices. Nevertheless it was possible to demonstrate a direct relation between protein content and pH of the pickle.

Introduction

This paper constitutes one of a series covering an investigation of factory-cured Wiltshire bacon. An outline of the complete investigation and the methods employed were reported in the first paper (7). Of the many factors that may affect the quality of bacon, only a limited number can be controlled in commercial practice. For instance, the salt, nitrate, and nitrite contents of the pickle, and certain curing practices, such as the method of pumping sides, and the curing time, are ordinarily controlled and standardized within a given factory. This gives some control over the composition of the product with respect to the curing salts. These constituents and practices have been dealt with in an earlier paper (8). Other factors which are not controlled to any extent in either the pickle or the product, except indirectly, may also have some influence on the quality of the bacon. These include the pH and oxidation-reduction potential of the pickle and product, the protein content of the pickle, and the moisture content of the bacon. This paper deals with measurements of these properties in bacon and pickle from different packing plants.

Certain evidence (4, 5, 9) indicates that the pH of both the pork and the pickles may affect the final quality of the bacon with reference to its water and salt content, colour, and subsequent taint development. Indirect inform-

¹ Manuscript received January 18, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 36 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 895.

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ation suggests that the oxidation-reduction potential may influence the colour. Brooks (2) has shown that, even in the absence of oxygen, the reaction between haemoglobin and nitrite yields a mixture of both nitrosohaemoglobin and methaemoglobin, and that the presence of a reducing agent is required to prevent the formation of the latter compound. Since methaemoglobin is not found to any extent in the interior of bacon, it follows that the tissues contain some reducing substance having an oxidation-reduction potential lower than that of haemoglobin-methaemoglobin (6).

Other results (3) indicate that the moisture content of the lean part of the bacon decreases as the salt content increases, even after due allowance has been made for the salt present. The protein content of the tank pickle may affect other properties, for example, rate of bacterial development and consequently nitrite production, and thus indirectly affect the quality of the final product. The results of these miscellaneous measurements are reported in this paper. The interrelations between the several constituents in, and properties of, the product will be discussed in a later article.

The detailed observations on the several properties were reduced by statistical treatment (12) and are presented in the same form as that used in an earlier paper (8).

Hydrogen Ion Concentration

The mean pH values over all samples of bacon and pickle, and their standard deviations on a single observation basis, appear in Table I. The results show that the pH of the bacon from the various sources was remarkably uniform, with a mean value of pH 5.74. In general, the pump pickles were

TABLE I
pH OF BACON AND PICKLE

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean	5.74	7.86	6.80	6.42
Standard deviation	0.14	0.58	0.67	0.21

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.0064	18	0.291	16	0.270	16	0.048
Between samplings	1	0.1023**						
Between sides within plants	22	0.0264**	17	0.379	15	0.646*	15	0.040
Between plants	21	0.0371						

* Indicates 5% level of significance in this and subsequent tables.

** Indicates 1% level of significance in this and subsequent tables.

alkaline, having a mean pH of 7.86. The tank pickle was slightly acid initially and became more acidic during cure. Even at the end of cure, however, it was still more alkaline than the sides. The variability in the pH of the pickles appears to be related to their protein content and buffer capacity, since spent pickle, having the highest protein content (Table V), is the least variable.

The results of an analysis of variance appear in the lower part of Table I. In spite of the small over-all variation in the pH of the bacon, the differences between samplings and between sides from the same plant were highly significant, while the differences between sides from different plants were not significantly greater than those observed within plants. Other investigations (4, 5, 11) have shown that there is considerable variability in the pH of pork. The findings suggest that the small variations in the pH observed in bacon may be due primarily to differences in the pH of the pork.

The difference between the pH of the pickles from different plants was significantly greater than the variations within plants for the cover pickle only. There was some evidence that there was a significant variation in the pH of the pump pickle in a given plant from time to time, but for reasons already given (7) it was impossible to distinguish precisely between variability originating from this source and that attributable to sampling and other errors.

Simple correlation coefficients were computed between the mean pH of the bacon over all samplings and the pH of the pickles and other known curing practices, as outlined in Table II. The three significant correlation coefficients obtained show that the pH of bacon is inversely associated with the days in cure and with the pH of the tank pickle \times days in cure, and directly related to the pH of the pump pickle. It appears that the number of days in cure is primarily responsible for the significance of the combined quantity.

TABLE II

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN THE pH OF PICKLE AND pH OF BACON

Quantities correlated	D.f.	<i>r</i>
Mean pH of bacon over all sides and samplings with:		
Pump stitches per side	20	0.00
Days in cure	20	-0.52*
pH of pump pickle (mean)	20	0.46*
pH of tank pickle (mean)	20	-0.09
pH of pump pickle (mean) independent of pH of tank pickle (mean)	19	0.49*
pH of tank pickle (mean) independent of pH of pump pickle (mean)	19	-0.20
pH of pump pickle (mean) independent of days in cure	19	0.32
Days in cure independent of pH of pump pickle (mean)	19	-0.41
pH of pump pickle (mean) \times stitches per side	20	0.09
pH of tank pickle (mean) \times days in cure	20	-0.50*

Partial correlative coefficients between the pH of the bacon and the pH of the pump pickle independent of days in cure, and days in cure independent of pH of pump pickle, were both insignificant. These results show that although both these factors influence the pH of the bacon, it cannot be said which is the more important.

Certain supplementary studies were therefore made to obtain more definite information. It was found that while the pH of pork was scarcely affected by immersion in pickles at pH 5.0 to 5.5 for periods of a day or two, the injection of relatively small quantities of acidic but unbuffered brines, comparable with pump pickle, had an immediate and permanent effect on the pH of the pork. This suggests that if it is desirable to modify the pH of pork by means of the pickle, acidification of the pump, rather than the tank pickle would be the more effective method.

It is known that the pH of pork varies considerably (4, 5, 11). Unpublished results indicate that under Canadian conditions it may range from pH 5.5 to 6.5. Since the pH of the bacon was 5.7 (Table I), it appears that the meat must have become more acidic during cure. This could scarcely have resulted directly from the injection or absorption of pickle, since the pickles were generally alkaline to pH 5.7. These considerations suggest that the extent of the reaction affecting the pH of the bacon is affected by the length of the curing period independent of the pH of the curing pickles.

Oxidation-reduction Potential

The methods employed for measuring the oxidation-reduction potential of both the bacon and pickle have already been described (7). These results were subject to some uncertainty for the following reasons: (i) the potentials observed in both bacon and pickle showed considerable "drift" with time, and although the readings were not accepted until equilibrium had apparently been established, it is probable that errors of the order of 5 to 10 mv. occurred in some samples; (ii) since the pickle samples were exposed to air during transport, sampling, and measurement, it seems likely that the observed potentials were somewhat higher than those occurring in the curing tanks; (iii) for bacon two apparently identical electrodes gave, on the average, widely different absolute potentials.

In spite of these uncertainties the results are reported in Table III, since certain deductions are possible. The values reported for bacon were obtained with the No. 1 electrode, which gave a mean potential of about 24 mv. as compared with a value of about -121 mv. obtained with the No. 2 electrode on the same samples. The results obtained with the No. 1 electrode are preferred since it reproduced, from time to time, the correct potential in a quinhydrone-buffer solution more closely and consistently than the No. 2 electrode.

All three pickles were found to have about the same mean potential. It seems probable that the observed values represent the actual potentials in

TABLE III
OXIDATION-REDUCTION POTENTIAL (Eh) OF BACON AND PICKLE

Statistic	Bacon (Electrode No. 1)	Pickle		
		Pump	Cover	Spent
Mean, mv. (+)	23.7	338	328	328
Standard deviation, mv.	89.9	31.8	30.2	35.6

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	31	2,489	18	975	16	554	16	628
Between samplings	1	9,025						
Between sides within plants	22	1,636	17	1,044	15	1,294	15	1,952*
Between plants	21	22,422**						

the pickles as received, as they were reproducible, within the error attributable to "drift", with different electrodes. Since these samples had suffered exposure to air, however, there is some doubt that they represent material at all comparable with that existing in a curing tank.

In order to obtain information on this point, the oxidation-reduction potential was measured daily at four positions in a tank during cure. The four positions represented two depths, 4 in. and 3 ft. 10 in. below the surface, at two places in the tank. These readings varied considerably with position, depth, and time, and further information is required before a definite statement can be made as to the effect of these variables. It is sufficient to point out that the maximum, minimum, and mean values observed were 245, -189, and 86.2 mv. respectively. Since these values are considerably lower than those reported for tank pickle in Table III, it would appear that a satisfactory estimate of the oxidation-reduction potential can only be obtained on samples that have not suffered undue exposure to air.

Although the observed potentials in the main series of experiments may not be absolute, the results of an analysis of variance are of interest. The difference between plants, for bacon and spent pickles, was the only statistically significant source of variance, distinguishable from experimental error. In this respect both the electrodes used in bacon yielded similar results. It appears from this that the oxidation-reduction potential of bacon is dependent on the handling and curing practices followed in a particular plant.

Because of the uncertainty of the results, only a few correlation coefficients were computed. The coefficient between the Eh of bacon (No. 1 electrode) and that of the tank pickle (mean of cover and spent) was insignificant ($r = 0.25$ for 19 degrees of freedom). Although the Eh of both spent pickle

and bacon differed significantly between plants, they were not significantly correlated ($r = 0.11$).

The results presented in an earlier paper (8) showed that the nitrite content of the bacon was related to the number of stitches used in pumping, independent of the nitrite content of the pump pickle. It was felt that this might be the result of the introduction of air or bacteria with the pump pickle. Since the introduction of air might affect the oxidation-reduction potential of the bacon, the correlation coefficient between this quantity and the number of stitches per side was computed. The insignificant value ($r = 0.03$) was obtained. This does not necessarily disprove the above hypothesis, as the measurements on the bacon were made a considerable time after the pumping operation.

Moisture Content of Bacon

The moisture content of the bacon was determined at all three samplings. The results, including the analysis of variance, appear in Table IV. The mean moisture content over all sides and samplings was 71.38% with a relatively small variation. Nevertheless, an analysis of variance showed that the differences between samplings and between sides from different plants were highly significant. The loss of weight during storage and smoking doubtless accounts for the differences between samplings, a subject to be discussed further in a later section of this paper. The differences between the moisture content of the sides from different plants may be the result of the differences in salt content (3). These relations will be presented in a later paper.

TABLE IV
MOISTURE CONTENT OF BACON

Statistic	
Mean, %	71.38
Standard deviation, %	1.58
Coefficient of variability	2.22

Analysis of variance		
Variance attributable to	D.f.	Mean sq.
Sampling and analytical error	86	0.621
Between samplings	2	93.968**
Between sides within plants	22	0.970
Between plants	21	3.100**

Protein Nitrogen Content of Pickle

The results of the Kjeldahl nitrogen determinations appear in Table V. Although expressed as the percentage of protein nitrogen this determination also includes lower compounds which yield ammonia on digestion. The mean protein nitrogen content of the pump pickle was 0.0008%. In some

instances, however, the samples contained almost as much as certain cover pickles. No statistical computations were made on the results obtained with pump pickles. The results in Table V show that the mean protein nitrogen content of cover pickle was about 0.09%, while that for spent pickle was 0.12%. The analysis of variance shows that the protein content of the pickles from different plants differs significantly, and probably reflects the effect of the different handling, treatment, or storage practice.

TABLE V
PROTEIN NITROGEN CONTENT OF PICKLE

Statistic	Cover	Spent
Mean, %	0.092	0.121
Standard deviation	0.049	0.040
Coefficient of variability	53.5	33.2

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error and variance between samplings	16	0.00032	16	0.00053
Between plants	15	0.00467**	15	0.00278**

Although the observed variations in tank pickle could be accounted for in this way, an attempt was made to determine whether the solubility of the protein was dependent on any other properties of the pickle. The computation of correlation coefficients between the protein content and the pH and salt content of the pickle yielded values of 0.63 (highly significant), and 0.25 (not significant), respectively. It is therefore concluded that, whereas the observed variations in the salt content of the pickle (8) have no effect, the solubility of the proteins increases with increase in pH within the range (Table I) experienced in practice. This latter finding is in general agreement with the results of other investigations (11).

Difference Between Samplings

It has already been pointed out (7) that the design of the experiment did not permit accurate estimation of the effect of time, as the observed difference between samplings included both systematic differences between the positions from which successive samples were taken, and the true effect of ageing. The results obtained with salt and nitrate (8) led to the conclusion that a systematic difference between positions did exist. In consequence the observed differences between the nitrite contents at the several samplings had to be attributed to the effect of position rather than time. The question therefore arises as to whether the observed changes in the pH and moisture content of the bacon

represent a real change with time or merely variation between different positions.

The mean pH and moisture content of the bacon at each sampling is given in Table VI. These results show that both the pH and moisture content decreased significantly between successive samplings. Since evaporation is to be expected during storage and smoking, the decrease in moisture content doubtless indicates a real effect of time rather than the influence of position.

TABLE VI
DIFFERENCES BETWEEN SAMPLINGS IN pH AND MOISTURE CONTENT OF BACON

Constituent or property	Mean by samplings			Remarks
	First	Second	Third	
pH	5.78	5.71	—	Differences between means highly significant
Moisture, %	72.83	71.39	69.91	Differences between any two means highly significant

The decrease in pH between samplings, although significant, is rather small, and might have resulted from the influence of position rather than a real change with time. Earlier results (Table II) indicate that the pH of bacon may be affected by the time in cure, and it is reasonable to believe that the pH might also be affected by the time from cure. The difference between the mean pH values at the different samples is not a satisfactory method of studying the effect of ageing, since the sides varied in age from 2 to 11 days at the time of the first sampling. Consequently, any change that takes place with time might reasonably have occurred to various extents in the sides, quite apart from the uncertainties arising from the effect of position.

In order to obtain more definite information on the effect of time, at various stages, on the pH of bacon, simple and partial correlation coefficients were computed between the pH of the bacon and the elapsed time before, during, and after cure. By making these computations separately for each sampling, the possible effect of differences between the positions from which successive samplings were taken was excluded. The results appear in Table VII.

Since the correlation coefficient between pH and days from slaughter to cure was not significant, it appears that the ordinary variations in cooling time have no effect on the acidity of the smoked bacon.

Since the pH of the pump pickle and the period in cure have been shown to affect the pH of bacon to some extent, it might be that the length of holding period prior to cure had an effect on the pH of pork which was subsequently altered by other factors. However, the results of investigations on rabbit and poultry muscle (1, 10), and some unpublished results on pork, indicate that the ultimate pH of muscle tissue is attained well within the shortest cooling period used in practice.

TABLE VII

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN pH OF BACON AND TIME IN CURE AND AGE FROM CURE

Quantities correlated	D.f.	<i>r</i>
pH of bacon—first sampling with:		
Days from slaughter to cure	15	-0.01
Days in cure	20	-0.53*
Days from cure to first sampling	19	+0.05
pH of bacon—second sampling with:		
Days in cure	19	-0.39
Days from cure to second sampling	19	+0.53*
pH of bacon—first and second samplings with:		
Days from cure when measurement made for both samplings	40	-0.09
pH of bacon—first sampling with:		
Days in cure, independent of days from cure to first sampling	18	-0.51*
Days from cure to first sampling, independent of days in cure	18	0
pH of bacon—second sampling with:		
Days in cure, independent of days from cure to second sampling	18	-0.40
Days from cure to second sampling, independent of days in cure	18	+0.53*

The correlation coefficients between pH and time in cure were negative, and significant at the first, but not at the second sampling. The coefficients between pH and time from cure were positive and significant only for the second sampling. Similar results were obtained when the opposing effects of time in cure and time from cure, were rendered independent by partial correlation. This indicates that the pH of bacon decreases during cure but increases during maturation.

The increase in the pH of bacon during maturation was confirmed by the results of a supplementary experiment in which ground samples of bacon were stored at 1 to 2° C. for some time. The final pH values attained varied from 6.0 to 8.3. It was also observed that the colour and final pH of the bacon appeared to be related. All samples at about pH 6.0 were grey, those at pH 7.0 greyish-brown to brown, while those at pH 8.0 or higher had retained their red colour. A further investigation of the effect of pH on colour change has been projected.

Difference Between Bacon and Pickle from Different Plants

In a previous paper (8) dealing with the chloride, nitrate, and nitrite contents of bacon and pickle, it was found that the differences between plants were usually the major source of variation. The properties and constituents considered in the present paper did not show such marked variation between plants. With respect to pH, significant differences between plants could be demonstrated for cover pickle, but not for the pump or spent pickle, or for the bacon. The oxidation-reduction potential measurements showed significant differences between the bacon and spent pickles from different plants.

The different practices followed in the different establishments were reflected by highly significant differences in the moisture content of the bacon, and the protein content of the cover and spent pickles.

The generalizations summarized in the above paragraph are evident from the results presented in earlier tables. Since individual plants seldom differed greatly from the general means, and since the factors considered in this paper could only be controlled indirectly, more detailed discussion of this source of variation is considered unnecessary.

Acknowledgments

The authors wish to express their appreciation of the assistance and co-operation of the individuals and firms referred to in the first paper, and in particular to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on statistical matters.

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CANADIAN WILTSHIRE BACON

IV. CORRELATION BETWEEN CONSTITUENTS AND PROPERTIES OF BACON¹

By W. H. COOK² AND W. H. WHITE²

Abstract

Simple correlation coefficients computed between several observed properties and constituents of factory-cured Wiltshire bacon showed the following to be associated: nitrate and chloride contents; nitrite and chloride contents; nitrite content and pH; and moisture and chloride contents. The first two of these associations probably arise from the pickle compositions and curing practices followed in the plants. The last two associations suggest a certain degree of dependence between the two properties, i.e., the nitrite content of the meat increases with the pH, and the moisture content decreases as the chloride content increases. When the moisture content of the bacon was expressed on a salt-free basis, the correlation between the moisture and chloride contents was not significant. This indicates that curing practices favouring a high salt content do not result in the removal of more moisture from the sides.

Introduction

The chloride, nitrate, nitrite, and moisture contents, pH values, and oxidation-reduction potential of factory-cured Wiltshire bacon and the pickles used for its manufacture, have been reported in earlier papers of this series (3, 4). These papers also reported the degree of correlation between similar constituents in the pickle and product. This paper deals with the correlations between the measured properties of the bacon.

Procedure

The methods employed for making these measurements have already been described (5, 8). The computation of correlation coefficients (7) between the observed quantities over 44 sides, representing 2 sides from each of 22 packing plants, served to determine whether statistically significant correlations existed between the measurements. It must be recognized, however, that a significant correlation between two quantities merely demonstrates that they are associated, and does not necessarily indicate that one is dependent on the other. Even where it seemed reasonably certain that one quantity was dependent on another, it was frequently difficult to determine which of the two was the causal agent. Thus a highly significant negative correlation between the pH and salt content of bacon could indicate either that the salt penetration was more rapid in the more acid sides, or that the pH decreases as the salt content increases.

¹ Manuscript received January 18, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 37 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 896.

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Results

Simple Correlation Coefficients

The simple correlation coefficients between these quantities appear in Table I. These coefficients were computed from the results obtained for each sampling at which the measurements were made, and also between the mean values for each constituent over all samplings for each of the sides analysed. This procedure was followed, because certain of the observed differences between samplings were attributable to the differences between positions (4, 5) from which successive samples were taken, while others may have represented a real change with time. In consequence it seemed desirable to compute the correlation coefficients from the results obtained at each sampling independently. On the other hand, the variations between different sides for certain properties were small. In such instances it was felt that the variance attributable to over-all sampling and experimental error for any two properties being studied might be sufficiently large to mask any existing correlation. Consequently it was desirable to use in addition the mean values for each measurement over all samplings in order to reduce the variance attributable to sampling and analytical error.

TABLE I

SIMPLE CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 42, for all except bracketted value = 30. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling			
	First	Second	Third	Mean of all
Nitrate in bacon with:				
Chloride in bacon	0.30*	-0.26	—	0.40*
Nitrite in bacon with:				
Chloride in bacon	0.45**	0.28	—	0.38*
Nitrate in bacon	-0.05	-0.23	-0.04	-0.07
Moisture in bacon	-0.04	-0.04	-0.06	-0.02
pH of bacon	0.56**	0.30*	—	0.47**
Eh potential of bacon (El. 1)	—	0.13†	—	0.16
Eh potential of bacon (El. 2)	[0.10]†	0.37*†	—	—
Moisture in bacon with:				
Chloride in bacon	-0.56**	-0.46**	—	-0.60**
Nitrate in bacon	-0.48**	-0.06	-0.07	-0.25
pH of bacon	0.18	0.17	—	0.13
Eh potential of bacon (El. 1)	—	0.05	—	—
pH of bacon with:				
Chloride in bacon	0.09	-0.23	—	-0.04
Nitrate in bacon	0.04	-0.05	—	—
Eh potential of bacon (El. 1)	—	—	—	0.26

† Correlated with mean nitrite content over all samplings.

* Indicates 5% level of significance.

** Indicates 1% level of significance.

It is evident that values for a number of possible correlation coefficients do not appear in Table I. Most of these omissions result from the fact that several of the determinations were not made at the third sampling. Since the observed oxidation-reduction potentials of the bacon are subject to some uncertainty for reasons already given (3), only a few computations relating this quantity to other properties were made.

The majority of the correlation coefficients reported in Table I are not significant and do not require comment. Significant positive correlations were obtained between the chloride and nitrate contents of the bacon at the first sampling and between the means over all samplings. These coefficients doubtless reflect the association between the concentration of these two substances in the curing pickles or between the curing practices used in different plants, and are therefore of little consequence.

A significant positive correlation was obtained between the nitrite and chloride contents at the first sampling, and between the mean nitrite and chloride contents over all samplings. There was also a significant positive correlation between the nitrite content and the pH of the bacon at all samplings.

Significant negative correlations were obtained between the moisture and chloride contents at all samplings, and between the moisture and nitrate contents at the first sampling. The only significant correlation involving the oxidation-reduction potential was obtained with nitrite at the second sampling, the No. 2 electrode being used. Since these measurements were uncertain, and those with Electrode 2 probably less reliable than those with Electrode 1 (3), further comment is unnecessary.

Partial Correlation Coefficients

The relation between the nitrite content and the pH and chloride content, and that between the moisture content and the chloride and nitrate contents, were investigated further by computing partial correlation coefficients between these quantities. The values obtained appear in Table II. The correlation coefficients between the nitrite and chloride contents, independent of pH, were positive and highly significant at both the first and second samplings independently, and also between the mean values for each side over all samplings. There is therefore little doubt that the nitrite content of these sides increased with their chloride content. It seems probable, however, that these two constituents are not dependent on one another but are merely associated through the pickle compositions or curing practices followed in the different plants.

The coefficients representing the degree of correlation between the nitrite content and pH, independent of chloride content, also indicate a definite association between these quantities, the nitrite content increasing as the pH increases. Certain evidence indicates that the pH of pork (2, 6) and the nitrite content of the bacon (4) are properties of the individual side and to that extent are independent of the curing practices followed. Consideration of these facts sug-

TABLE II

PARTIAL CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 41. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling		
	First	Second	Mean
Nitrite in bacon with chloride in bacon independent of pH of bacon	0.48**	0.59**	0.45**
Nitrite in bacon with pH of bacon independent of chloride in bacon	0.59**	0.39**	0.53**
Moisture in bacon with chloride in bacon independent of nitrate in bacon	-0.50**	-0.49**	-0.56**
Moisture in bacon with nitrate in bacon independent of chloride in bacon	-0.39**	-0.21	-0.01

**** Indicates 1% level of significance.**

gests that there may be a dependence of one of the properties on the other rather than a mere association. If this is so it seems likely that the nitrite content is dependent on the pH rather than the reverse, since it is highly improbable that the small quantities of nitrite present would have any effect on the pH of the bacon. Such a dependence could be explained in several ways, namely: a more rapid penetration of the nitrite from the tank pickle as the pH of the meat increases; a more rapid production of nitrite from nitrate at higher pH levels; or a decreased rate of combination of nitrite with the muscle proteins and pigments at high pH levels. It has been shown that a high electrical resistance is associated with a high pH, and a slow penetration of chloride (2). If the absorption of nitrites is comparable with that of chlorides it seems unlikely that the first explanation is adequate for the observed differences in pH. Likewise it would appear that the rate of combination of nitrite with the muscle pigments would not be affected appreciably (1) by the variations in pH at the levels observed (3) in these sides. On the other hand the rate of reduction of nitrates by bacterial activity might reasonably proceed more rapidly as the pH increased within the observed range.

The results in Table II show that the correlations between moisture and chloride contents of the bacon, independent of nitrate content, were highly significant at each sampling independently, and for the mean values over all samplings. These show quite definitely that the moisture content decreases as the chloride content decreases. The correlation between the moisture and nitrate contents, independent of chloride content, was significant only for the results of the first sampling. It appears therefore that although the nitrate content may have a slight independent effect on the moisture content of the bacon, it is of secondary importance compared with that of chloride.

Relation between Moisture and Chloride Contents

The decrease in the moisture content with increase in chloride content of the whole bacon may merely reflect the effect of the additional dry matter present as sodium chloride. On the other hand it may be due to this effect plus the influence of certain curing practices, if conditions favouring the absorption of greater quantities of chloride also favour the loss of additional quantities of moisture from the sides. In order to eliminate the direct effect of the chloride on the dry matter content, the moisture contents, as previously reported (3), were computed on a sodium chloride-free basis for each side. When expressed as such, the mean moisture content was found to be 74.03% with a standard deviation of 0.70%, as compared with a mean of 71.38% and a standard deviation of 1.58% (3) calculated on the basis of the whole bacon. It is therefore evident that the direct effect of variations in the chloride content was a major source of variation in the moisture content of different sides.

The correlation between moisture, on a salt-free basis, and sodium chloride content was not significant ($r = 0.23$, for 40 degrees of freedom). The negative relation between the chloride and moisture contents of whole bacon, noted previously, was therefore due entirely to the additional dry matter contributed by the sodium chloride. These findings, together with the fact that the moisture content of bacon on a sodium chloride-free basis was essentially the same as that of fresh pork, indicate that the net result of pumping and curing is to increase the salt content, with little, if any, loss of moisture.

Acknowledgments

The authors wish to thank the individuals and firms referred to in the first paper of this series for their able assistance and co-operation, and in particular to Messrs. A. E. Chadderton and E. A. Rooke, laboratory assistants, National Research Laboratories, who were responsible for most of the computations.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

MAY, 1940

NUMBER 5

ON THE LIFE CYCLE AND OTHER ASPECTS OF THE SNAIL, *CAMPELOMA*, IN THE SPEED RIVER¹

By J. C. MEDCOF²

Abstract

Judged from a statistical study, the life span of the snail is five years. Parturition occurs in summer and year classes are recognizable. Sizes of approximately 12, 17, and 23 mm. are reached in one, two, and three years respectively. Sexual maturity is reached in two years and reproduction is parthenogenetic. Some snails hibernate and some aestivate. Rest marks on shells and opercula and a limy deposit in the protoconch were studied.

In 1934 the writer's attention was directed by Mr. J. G. Oughton of the Royal Ontario Museum of Zoology, Toronto, to a colony of a single kind of freshwater snail, *Campeloma*, living in the Speed River at Hespeler, Ontario. The specific identification of the form is undecided because no careful taxonomic study of the genus has been made yet. Mr. F. C. Baker of Urbana, Illinois, has examined collections and states that the form is allied to *C. decisum* but that it is probably a new species.

Successive random samples of the snails were collected from the river on July 9, 1934, November 11, 1934, February 3, 1935, and March 8, 1936, and the shell heights measured to the nearest millimetre with calipers. A separate record was kept of the measurements of empty shells occurring in the samples except in 1935, when they were not made. The results of the measurements are presented as size-frequency distribution curves, plotted on a percentage basis, in Fig. 1. Only two curves have been prepared from the measurements of the empty shells—one for the two 1934 samples combined and the other for the 1936 measurements alone.

In their study of *C. rufum* in the Salt Fork River, Illinois, Van Cleave and Altringer (7) have justified the treatment of clear modal groups such as appear in the present curves as year classes. The present data are considered in the same way and summarized in Table I. Three maxima are usually apparent in each curve.

GROWTH RATE

Table I was used as a basis for constructing the growth curve, Fig. 2, by merging the data for the separate year classes represented. There seems to have been little variation in the growing conditions during 1934 and 1935,

¹ Manuscript received January 23, 1940.

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so this procedure seems justified. Although the largest reported Salt Fork River shells measured 40 mm. and were considered three years old, the writer has found Speed River shells as large as 47 mm. It seems improbable from the growth curve that this size could be attained in the Speed River in less

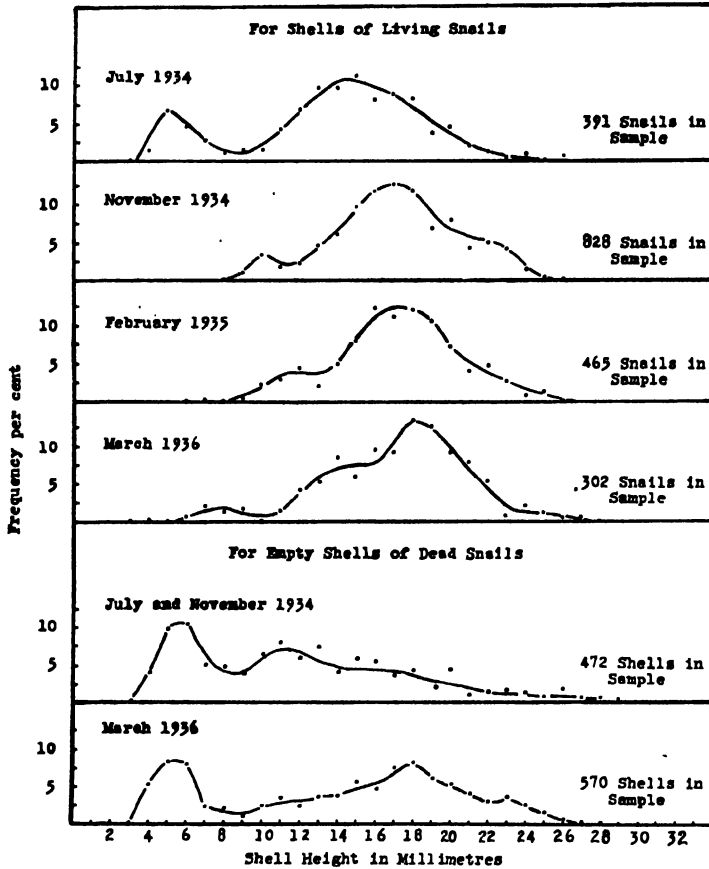


FIG. 1. Size-frequency distribution of heights of snail shells.

TABLE I
SHOWING MODAL VALUES TAKEN FROM FIG. 1

Date of collection	First maximum, mm.	Second maximum, mm.	Third maximum, mm.	Fourth maximum, mm.
July 1934	6	15	19	—
Nov. 1934	10	17	23	—
Feb. 1935	12	17	—	—
Mar. 1936	13	17	—	—
<i>Empty Shells</i>				
1934 collections	6	12	18	—
1936 collection	5	11	18	23

than five years. The northern form, then, grows more slowly, lives longer, and reaches a greater ultimate size than the Salt Fork River snail.

WINTER CONDITIONS

Fig. 2 shows that in the Speed River colony there is no slackening in the growth rate during the first winter, but that in the second there is a marked depression. The reason for this pause may be that at this time the two-year-old snails are developing their first brood of uterine young. Reference to the frequency curves for empty shells (Fig. 1) shows evidence suggesting that the winters are particularly hard on the snails. A high mortality among the newly born is indicated by the maxima at 5 and 6 mm. The writer has not attributed this to any particular cause. The other three modal values, however, at 11, 18, and 23 mm., if marked on the mean growth curve (Fig. 2) will each be found to have a corresponding "winter" position on the abscissal time scale. Winter killing is probably largely responsible for the multimodal nature of the frequency curves for empty shells.

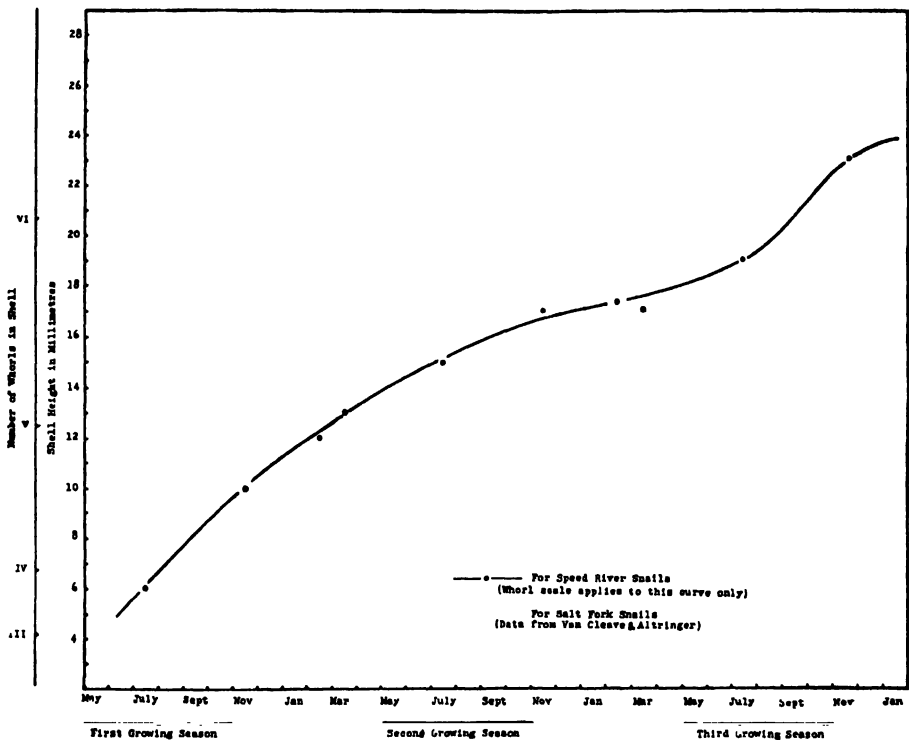


FIG. 2. Growth rate of shells.

Call (2) has said that in the fall of the year the snails (*Campelema*) bury themselves deeply in the mud and hibernate until spring. Van Cleave and Altringer found that the Salt Fork River stock remained active all winter long. This apparent disagreement illustrates the flexibility of the animal's

behaviour, because the present study corroborates both observations. In the stream it was found that throughout the winter the snails continued to crawl about over the hard bottom and could be collected easily by clearing away the ice. On the same days *Campeloma* in the pond were found to be buried to some depth in the bottom, as will be described.

Above the point in the river where the sampling was done there is a mill pond, a hundred acres or more in extent, held back by a dam. The pond bottom here is of soft black muck often smelling strongly of hydrogen sulphide. Throughout the warmer parts of the year there is a congregation of snails (*Campeloma*) on the bottom beginning at the pond margin and extending out to a depth of 10 or 12 in. Here the snails are usually so plentiful that it is seldom more than an hour's work to collect 200 or 300 of them. (The reason for this summer congregation is obscure. It may be related to oxygen depletion of the water at greater depths owing to decomposition of the bottom muck.) Strangely enough, by November 10, 1935, when the surface water temperature was 6.8° C., these snails had completely disappeared from the margin of the pond but were found at greater depths by dredging from a boat. During the winter they were secured by dredging mud through a hole in the ice. After some exposure to air a number of the latter extruded their young.

On March 24, 1935, the writer was fortunate to be at the pond during the period of emergence from hibernation. The surface water temperature was 6.6° C., the ice had "gone out" on March 17, and March 22, 23, and 24 were all bright warm days. On this day there were very few snails to be found about the pond margin, but several were collected from 2 or 3 ft. of water. Many of these were crawling towards shore. In each case the pubescent covering of the shell and the operculum were impregnated with an adhesive layer of fine silt. Besides this the periostracum and even the pearly layers of the shell itself were dyed by the black muck. This dark colour persisted even after thorough washing and scrubbing and after months of preservation. None of these peculiarities are to be found in shells taken from the pond at other times of the year and never in those taken from the river itself. The shells must be bleached out to their normal greenish colour during the spring, for by July they resemble the river shells in every detail. It seems reasonable to attribute the change to hibernation burial in the muck of the pond bottom.

AESTIVATION

Aquatic pulmonates are known to practise aestivation and to form epiphragms in the mouths of their shells as do their terrestrial relatives (3). Aestivation is much less common among the larger branchiate types. Mr. William LeRay of the University of Toronto recounts that in August 1927 he found *Campeloma* in a vigorous condition along with crayfish under stones in the dry bed of a brook that normally flows into the Crane River near Johnston's Harbour, Ont. It is unusual to find *Campeloma* except in permanent waters, but it would seem that it can survive in temporary brooks for some time.

REACTION TO CHANGE IN WATER LEVEL

An example of striking contrast in behaviour of the snails in different habitats may be cited. Rapid changes in the water level of the Speed River pond are frequently brought about by the varying needs of the mill and by other factors. These changes affect the pond snails during the summer, for they were observed to travel back and forth maintaining a position just at the water's edge. In some cases they were observed to travel as much as 12 ft. overnight in places where the beach sloped gently.

The opposite extreme was found among snails living on the flat surfaces of rocks below the dam. Here the stones were completely overgrown with a dense mat of *Cladophora* except for small circular patches which were covered by the extended feet of snails. The shells of these animals themselves bore a heavy growth of *Cladophora* and it appeared that they remained permanently attached to the small bare patches of rock. On one occasion marked animals were observed over a period of two days throughout which they did not leave their positions.

PARTHENOGENESIS

No male of the species was found during this study, although the genitalia of approximately 450 snails were examined. Mr. J. G. Oughton has made a similar report on the results of his own earlier study of the Speed River snails. Thus it appears that parthenogenesis is the normal form of reproduction in the Speed River colony as in the Salt Fork River stock (5, 7).

SEXUAL MATURITY AND REPRODUCTIVE CAPACITY

The smallest pregnant snail found during the investigation measured 17.5 mm. in height, contained one embryo, and was collected in February 1935. From Fig. 2 it would appear that, at the earliest, reproduction begins either during the latter part of the second summer or during the second winter. Probably in most cases it is delayed until the third summer, when the length is 19 mm. It is at about this same size or slightly larger that the Salt Fork River snails begin to reproduce, but they are then only one year old. The northern form seems to have a delayed sexual maturation.

TABLE II

THE RELATION BETWEEN SIZE, AGE, AND REPRODUCTIVE ACTIVITY OF 55 SNAILS COLLECTED IN FEBRUARY AND MARCH 1935

Age of snail, years	Size of snail, mm.	No. of this class pregnant, %	Average number of eggs and/or embryos in pregnant uteri
1	5 - 12	0	0
2	13 - 18	18 (high)	2
3	19 - 23	77	6
4	24 - 28	93	15

Table II summarizes the results of uterine examinations made on snails collected early in the spring before parturition began. There is a clear, directly proportional relation between the size of the parent and her reproductive capacity. The highest fertility record, 22 mature and 4 developing embryos, was that of a 28 mm. snail taken from the mill pond on March 24, 1935. A study of Table II with reference to Fig. 1 will demonstrate that the maintenance of the colony depends almost wholly on the reproductive activity of the three-year-olds. The stock of *C. rufum* in Illinois, as shown by Van Cleave and Altringer, is more prolific and probably maintains itself chiefly through reproduction by the two-year-olds.

Table III shows the results of uterine examinations made on snails collected at different times of the year. From this it seems that ovulation and embryonic development go on at all seasons but that parturition is intermittent and occurs only in the summer. In other words, the mature embryos accumulate in the uterus during the winter. These conclusions are much the same as those of Van Cleave and Altringer.

TABLE III

THE RESULTS OF UTERINE EXAMINATIONS OF 73 SNAILS 20 MM. OR MORE IN HEIGHT

Date of collection	Number examined	Empty, %	With eggs or very small embryos, %	With large embryos, %
July 1934	21	33	48	24
Nov. 1934	15	20	60	67
Feb. and Mar. 1934	37	11	78	89

REST PERIODS IN SHELL DEPOSITION

Careful measurements of the height, width, and number of whorls of shells and the heights and widths of the corresponding opercula have shown that there is a precise relation among all these. It has therefore been possible to supply two size scales to the growth curve (Fig. 2), one showing the shell height and the second showing the number of whorls.

Practically all snails more than one year old show at least one heavy brown line paralleling the growth lines somewhere on their shells. The opercula too, ordinarily show one or more concentric rings that stand out more clearly than the growth rings. A study involving the measurements of the relative positions of these heavier lines on the shells and opercula has convinced the writer that the two are formed simultaneously.

The peculiar conditions that favour the formation of these lines have not been discovered. However, a number of specimens that failed to grow appreciably while in a laboratory aquarium for eleven months invariably showed a broad dark brown band at the edge of the shell. It seems likely that line-formation is associated in nature with rest periods in shell deposition, but the occasion for these periods is still in doubt.

SHELL EROSION AND STRUCTURE OF THE SPIRE TIP

A large shell dropped into weak acid begins "gassing" at the protoconch immediately but only there, showing that in this oldest region of the shell the limy part is least protected by the periostracum. Shell erosion in the Speed River colony was conspicuously absent, probably because the stream flows through country where the main outcrop is limestone. In some habitats, on the contrary, several of the uppermost whorls may be completely missing from old shells, which may be scarred additionally with deep furrows or pits scattered over other parts.

The writer was curious to know if this removal of the upper whorls inconvenienced the snail and how repairs were made to the shell when it was perforated by erosion. To study this, median vertical sections were made of several shells. It was discovered that although the newly-born snail occupied all the whorls of its shell, it very soon began to fill in the smallest ones with a solid limy deposit. This process appeared to be continuous, a total of 0.7, 1.7, 2.4, and 3.0 whorls being completely filled in by the time the snail reached 10, 20, 30, and 40 mm. in height respectively. The fact that the snail normally abandons the cavity of the protoconch suggests that erosion of the solid part of the spire would not interfere seriously with the life processes.

A microscopic study of thin sections of the shell showed this central secretion to be structurally distinct from the three typical layers. The new deposit is probably secreted by that part of the mantle covering the visceral hump, while the periostracum, ostracum, and hypostracum seem to be formed at the mantle margin (6). With this probable difference in origin it might be expected that the structure would be different. It seems likely that a secretion of a similar type would be useful in making repairs to the shell when it is perforated by erosion.

REVERSAL OF SHELL SYMMETRY

Reversed shells are not infrequently met with in this genus but in the Speed River stock they are rare. Of 530 uterine embryos examined 2 were sinistral, and in a sample of 870 snails above the age of one year, only 2 were sinistral. This is low compared with the Salt Fork River variety (4).

ENEMIES OF *Campeloma*

Baker (1) records several fish, amphibian, reptilian, and bird enemies of *Campeloma*. The writer has no direct evidence of fish preying upon the snail in the Speed River, but he has examined only five stomachs, from two common suckers, two bullheads, and one dace, all large enough to take snails. The writer examined the stomachs of nine bullfrogs (*Rana catesbiana*) from the pond and found that while tadpoles and small frogs constituted a greater part of the diet, snails (*Campeloma*) of various sizes formed an estimated 24% of the bulk of the stomach contents.

Only one observation was made on birds as possible enemies and this was not in the Speed River but near Burnt River at Coboconk, Ont., June 9, 1934.

Freshly regurgitated pellets of the European starling were found at the base of a dead pine tree where the birds were nesting in what had been the nest of a flicker. The pellets contained three snails (*Campeloma*) measuring 20, 22, and 26 mm. in height. None of these had been digested, for the bodies and opercula were still intact within the shells.

A MITE IN THE MANTLE CAVITY

Living in the mantle cavity of the snail was a mite, kindly identified by Miss Ruth Marshall as *Unionicola campelomaicola*. In the river proper, 18% of the snails were infested but rarely was there more than one mite in a snail. In the pond stock there was a 46% infestation with an average incidence of 1.6 mites per infested snail.

Besides the above, an insect, *Mesovelia bisignata* (Uhler), identified by Dr. J. D. Detwiler of the University of Western Ontario and regarded as a visitant, was found in the mantle cavity of one snail. In another, one specimen of the predatory leech *Helobdella nepheloidea* (Graf), identified by Dr. J. P. Moore of the University of Pennsylvania, was found.

A FLUKE IN THE UTERUS

In the uteri examined during the embryo counts many metacercaria were discovered. There were more in snails collected during the summer than at other times of the year and more in the snails living in the pond than in those taken from the river. The highest infestation was recorded for July 1934, when it reached 88% with an average incidence of 29 flukes per infested animal. The smallest snail dissected in this study was 15 mm. high and it contained five flukes.

Acknowledgments

The writer is indebted to the University of Western Ontario for the provision of the necessary facilities for the conduction of this study; to Professor A. D. Robertson, Head of the Department of Zoology of the University, for the direction of the work during the two years it was carried on; and to Mr. J. G. Oughton of the Royal Ontario Museum of Zoology, for assistance and advice from time to time.

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STUDIES ON *STRONGYLOIDES AGOUTII* SP. NOV. FROM THE AGOUTI (*DASYPROCTA AGOUTI*)¹

BY HENRY J. GRIFFITHS²

Abstract

A morphological and biological study is presented of a hitherto unrecorded member of the genus *Strongyloides* from the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The name *Strongyloides agoutii* sp. nov. is proposed for this species.

Observations on the free-living development over a period of three years showed the indirect type to prevail; no seasonal variation was observed. Continuous propagation of the free-living generation of this species was not observed in faecal cultures or on artificial media.

A brief résumé of the classical studies on species of the genus *Strongyloides* is included, together with a summary of existing hypotheses and theories on the biology of this group. A list of species and hosts for this genus is given.

Introduction

As a general rule, a bisexual parasitic generation alternating with a larval stage outside the host may be considered as characteristic of the mammalian nematode parasites. This larval stage is essential and is subject to many modifications depending upon the adaptations of the parasite concerned. The non-parasitic phase of the life cycle of *Strongyloides* is similar to that of many other intestinal nematodes in that the offspring of the parasitic generation may develop directly into forms infective to a new host. In addition to this, however, *Strongyloides* may produce a true free-living generation consisting of adult rhabditiform males and females, which in turn reproduce sexually and give rise to filariform larvae infective to a new host.

The occurrence of this true free-living generation is uncommon among nematode parasites, and the genera *Strongyloides* and *Rhabdias*, in which it is found, are differentiated from other nematodes of vertebrates by this biologically significant mode of development. Another distinguishing feature of these two genera is the fact that the parasitic stage in the intestine of the host is represented only by female worms, considered by some investigators to be parthenogenetic and by others hermaphroditic. For many years the validity of this fact was unquestioned until Kreis (23) and Faust (9) reported the occurrence of parasitic males in infections of *S. stercoralis* in man and dogs.

The genus *Strongyloides* presents excellent opportunity for detailed study of both the parasitic and free-living forms of nematodes, and offers problems in nematode biology of an intricate and complex nature.

¹ Manuscript received September 26, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

² Assistant.

The studies on the genus *Strongyloides* presented in this paper were undertaken to throw further light on certain inconsistencies recorded in its life history. The free-living and parasitic phases of a hitherto unrecorded strain of *Strongyloides* were observed. The results were compared with those obtained by workers investigating other species.

Materials and Methods

The supply of *Strongyloides* material was provided by the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The animals were kept indoors in wooden frame cages covered with light wire fencing. Each animal was supplied with a sleeping box and bedding of wood shavings, about 2 in. deep. The cage floor was of asphalt, which permitted easy washing and disinfection. The maintenance ration consisted of bananas and coarse rolled oats supplemented by sprouted grain, peanuts, carrots, sweet potato, or apple when available. A small amount of cod liver oil was mixed occasionally with the rolled oats as a source of vitamins A and D.

The room in which the agouti were housed was held throughout the year at a temperature of about 70° F. As the floor of the pens was covered with shavings and as the bedding was usually dry, any possibility of the propagation of various species of free-living nematodes, the introduction of which might have caused considerable confusion, was eliminated.

Owing to the rapid development of the free-living phases of *Strongyloides*, it is very desirable that faeces for culturing be collected and used as soon after passage as possible. The agouti was observed to defaecate at a fairly regular period after feeding and usually in the same location in the cage, so that collection of fresh faeces was a minor problem. When in captivity and fed on the diet detailed above, the agouti passes stools that are moist and solid.

The customary Petri dish, 100 by 15 mm., was found satisfactory for the routine faecal culture observations of the free-living phase of development. On account of the heaviness of infection of the agouti, it was not necessary to culture large quantities of faeces. If too much was used, difficulty was experienced in making accurate observations under the binocular dissecting microscope because of the density of the culture. Cultures were mixed to a semi-solid consistency and kept adequately moistened. During the early stages of observation the cultures were occasionally stirred if a fungus growth appeared. For certain observations the faecal material was mixed with approximately one-half its volume of animal charcoal.

All cultures, unless otherwise stated, were incubated at room temperature, usually 22° to 25° C., but with a possible range of from 15° to 30° C. To prevent undue drying of the cultures during incubation above 25° C., the Petri dish lids were lined with filter paper, which was readily moistened. At temperatures over 30° C., it was more satisfactory to place the cultures over water in large covered culture dishes.

The free-living and parasitic forms of *Strongyloides* were killed and fixed by various methods, and although much care and attention were given to methods employed, considerable shrinkage and distortion of the parasitic forms occurred. Specimens were killed and fixed in hot 70% alcohol containing 3 to 5% glycerine. The container was loosely covered to exclude dust and set aside until the alcohol had evaporated, thus leaving the worms in dilute glycerine, suitable for microscopic examination. This method was found to be quite satisfactory. Hot 70% alcohol was not found to be as satisfactory a fixative as the alcohol and glycerine. Most satisfactory results were obtained by killing the specimens in hot water, fixing in 5% formol-saline, washing, and gradually transferring to glycerine alcohol. Material may be stored in 70% alcohol containing 3 to 5% glycerine for a considerable time; the presence of glycerine is a safety measure against desiccation owing to the evaporation of the alcohol. The ova of *Strongyloides* can be preserved in 5% formol-saline for several months without shrinkage or appreciable distortion.

Remarks on the Genus *Strongyloides*

The homogeneity and simplicity in structure of the members of the genus *Strongyloides* may be considered responsible for the difficulties that have arisen concerning the systematics of this group. Species have been recorded from a wide variety of unrelated hosts, as shown by the following list:

LIST OF KNOWN SPECIES OF THE GENUS *Strongyloides* GRASSI, 1879

Type species: *Strongyloides stercoralis* (Bavay, 1876), from man.

Other species:

- S. akbari* Mirza and Narayan, 1935, from *Crocidura coerulea*—India.
- S. avium* Cram, 1929, from domestic fowl—United States.
- S. canis* Brumpt, 1922, from dog—Japan and China.
- S. cebus* Darling, 1911, from *Cebus hypoleucus*—United States.
- S. chapini* Sandground, 1925, from *Ilydrochoerus hydrochoera*—United States.
- S. felis* Chandler, 1925, from cat—India.
- S. fülleborni* von Linstow, 1905, from *Anthropopithecus troglodytes* and *Cynocephalus babuin*—Africa.
- S. longus bovis* de Gaspari, 1912, from ox—Turin.
- S. minimum* Travassos, 1930, from *Dafila bahamensis*—Brazil.
- S. mustelorum* Cameron & Parnell, 1933, from *Mustela ermina*—Scotland.
- S. myopotami* Artigas & Pacheco, 1933, from *Myopotamus coipus*.
- S. nasua* Darling, 1911, from *Nasua narica panamensis*—Panama.
- S. ophidae* Pereira, 1929, from *Drimobius bifossatus*.
- S. oswaldoi* Travassos, 1930, from domestic fowl—Brazil.
- S. ovocinctus* Ransom, 1911, from *Antilocapra americanus*—United States.
- S. papillosus* (Wedl., 1856) Ransom, 1911, from sheep, goats, rabbits, etc.
- S. pereirai* Travassos, 1932, from *Elosia rustica*—Brazil.
- S. ransomi* Schwartz & Alicata, 1930, from pig—United States.

- S. ratti* Sandground, 1925, from *Rattus norvegicus*—United States.
S. simiae Hung and Hoeppli, 1923, from *Macaca* sp.
S. stercoralis var. *eryxi* Mirza & Narayan, 1935, from *Eryx johnii*—India.
S. stercoralis var. *vulpi* Mirza & Narayan, 1935, from *Vulpex alopex*—India.
S. suis (Lutz, 1894) von Linstow, 1905, from pig.
S. venezuelensis Brumpt, 1934, from *Rattus norvegicus*—Venezuela.
S. vituli Brumpt, 1921, from ox—France.
S. viviparous (Probstmayer, 1865) von Linstow, 1905, from horse—Europe.
S. westeri Ihle, 1917, from horse—Holland.

In addition the following undetermined species of *Strongyloides* are listed by Sandground (28):

- Strongyloides* sp. from lemur, Weinberg & Romanovitch, 1908.
Strongyloides sp. from fox, Romanovic, 1914.
Strongyloides sp. from guinea pig, Krediet, 1921.

Hall (20) makes reference to *Strongyloides* sp. from a rodent, but he has been unable to locate any reference to a paper by Parona whereby the observation might be confirmed.

There has been much difference of opinion as to the validity of many of these species. The difficulty of observing and recording any distinct characters on which specific distinction may be made, has led to a tendency on the part of many workers to erect new species on the grounds of the occurrence of the parasite in a previously unrecorded host. In other cases, the description of new species has been based almost entirely on dimensions of the body. These distinctions, probably legitimate in many cases, have seldom been accompanied by drawings that would be of benefit to subsequent investigators. Until recent years, there has been a tendency to neglect the bisexual forms when describing new species. In many species, the free-living forms are remarkably similar in appearance and do not possess distinctive features by which they may be characterized. However, differences do exist in the morphology of the bisexual generation, and in at least one species, *S. fülleborni*, these characters are sufficient for species identification. Goodey (15) has shown that constant differences of structure do exist between the free-living stages of *S. fülleborni* and *S. stercoralis* and that the free-living male of *S. ratti* materially differs from the free males of the other two species mentioned. These observations have been made only by examination of the species in question in as great detail as possible, together with careful camera lucida drawings. Looss (26, p. 215), who has furnished the best and most detailed drawings of *S. stercoralis*, writing on the occurrence of the genus in animals states: "Besides being found in man, species of *Strongyloides* are very frequent in animals (mammals, birds and reptiles); so far as my personal observation goes, they so greatly resemble *Strongyloides stercoralis* that it is difficult to say whether they are the same species or not. The free-living generations on the contrary, so far as I am acquainted with them, show

slight but distinct differences from one another, which make it probable that different species exist."

Chandler (4) has undertaken the comparison of representatives of the genus from monkeys, cats, and man with the published characters of other forms available at that time. He suggests that the forms of *Strongyloides* considered in his paper be grouped into two species, *S. papillosus* and *S. stercoralis*, the species *ovocinctus*, *fülleborni*, *suis*, *simiae*, and *cebus* being reduced to hostal varieties or sub-species of *papillosus*, and the species *nasua*, *canis*, and *felis* to varieties or sub-species of *S. stercoralis*.

It would appear then, that the validity of many species of *Strongyloides* is questionable. Species differentiation has in many cases been based on proportionate lengths of different parts of the body without morphological distinctions. The observations of many investigators show that there may be a very wide range of variation in these ratios even between individuals in the same host, and there is little doubt that unless accompanied by other significant morphological features, they are of no value for specific diagnosis.

At the present time, however, it would seem that lack of data prevents a systematic reorganization of the genus on a morphological, physiological, or biological basis.

Strongyloides agoutii sp. nov.

The creation of numerous species of *Strongyloides* may be unwarranted, yet, when the basis for the taxonomy of this group is eventually laid, such species can be rendered invalid with greater ease and fewer complications than if they had originally been grouped under one specific name. On these grounds, it is considered advisable to create a new species for the strain of *Strongyloides* found in the agouti (*Dasyprocta agouti*).

A summary of the more important dimensions of 25 specimens of the parasitic and free-living generation is given in Table I. All measurements were made on unpreserved material. Specimens were examined in water and were rendered motionless by the careful application of heat.

Parasitic Generation

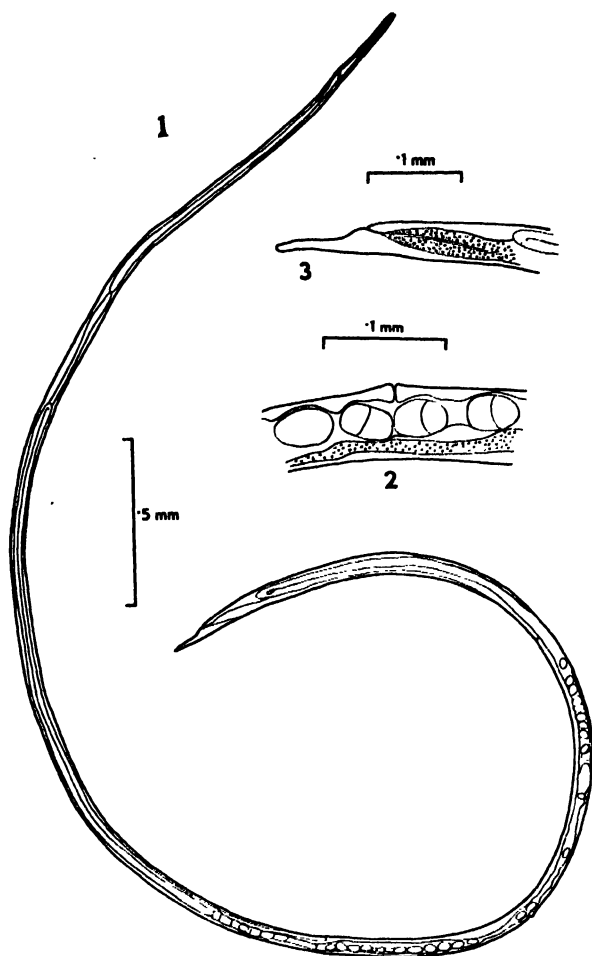
Female (Fig. 1). The length varies from 3.94 to 6.45 mm., and the width from 0.03 to 0.059 mm., measured at the middle of the body. The body is long and filiform, showing equal thickness from the base of the oesophagus to the region of the posterior ovarian loop. The cuticle is exceedingly finely striated, the striations being most easily seen in the tail region. From the base of the oesophagus, the body gradually becomes attenuated anteriorly to a diameter of about 0.015 to 0.020 mm. The mouth is small and leads directly into the oesophageal lumen. It consists of three indistinct lips with two projections on each, which are presumably papillae; the minute size of these structures renders them exceedingly inconspicuous. The first quarter of the oesophagus is slightly more muscular than the rest; a slight enlargement was observed at the posterior end of the first region. The oesophagus gradually increases in size posteriorly and is from 0.975 to 1.450 mm. long.

TABLE I
MEASUREMENTS OF 25 SPECIMENS OF *Strongyloides agoutii* SP. NOV.

	Range (n = 25), mm.	Mean, mm.	Standard deviation*	Coefficient of variation, %
<i>Parasitic female</i>				
Total length	3.94 - 6.45	5.170	0.7242	14.0
Width at middle of body	0.03 - 0.059	0.044	0.0063	14.3
Length of oesophagus	0.975 - 1.450	1.210	0.1359	11.2
Length from vulva to tip of tail	1.275 - 2.300	1.750	0.2606	14.8
Length of tail	0.075 - 0.109	0.091	0.0099	10.8
Ova—length, μ	46 - 58	51	3.926	7.6
width, μ	23 - 29	26	1.706	6.5
<i>Free-living male</i>				
Total length	0.870 - 1.050	0.965	0.0617	6.3
Width at middle of body	0.050 - 0.070	0.058	0.0067	11.5
Length of oesophagus	0.120 - 0.160	0.135	0.0096	7.1
Length of tail	0.075 - 0.100	0.086	0.0085	9.8
Length of spicules (10 specimens only)	0.028 - 0.035	0.031	0.0024	7.7
Length of accessory piece (5 specimens only)	0.018 - 0.023	0.021	0.0019	9.0
<i>Free-living female</i>				
Total length	0.975 - 1.350	1.126	0.0930	8.2
Width immediately anterior to vulva	0.045 - 0.075	0.062	0.0075	12.0
Length of oesophagus	0.140 - 0.188	0.156	0.0126	8.0
Length of tail	0.090 - 0.165	0.123	0.0170	13.7
Ova—length, μ	38 - 62	47	4.980	10.5
width, μ	28 - 40	36	3.187	8.8
<i>Filariform larva</i>				
Total length	0.645 - 0.760	0.710	0.0365	5.1
Width at junction of oesophagus and intestine	0.018 - 0.022	0.020	0.0012	6.0
Length of oesophagus	0.276 - 0.354	0.322	0.0252	7.7

$$* \sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}.$$

The longest oesophagus does not necessarily occur in the longest worm, and it may be said that the oesophagus varies from one-quarter to one-fifth of the total length of the body. The vulva (Fig. 2) possesses prominent lips and is situated well posterior to the middle of the body at from 1.275 to 2.300 mm. from the tip of the tail. The gonad is double and consists of an anterior and posterior loop, which may be of the simple hair-pin bend type or may be twisted. No constancy in this character was observed in the material examined. Some specimens showed the simple type in both uteri, while a few showed both loops twisted and others only one loop twisted. The ovaries and uterus occupy most of the body cavity, the bends of the former occurring close to the oesophageal and anal ends of the intestine. Between the posterior ovarian loop and the anus the body commences to taper, becoming consider-



FIGS. 1-3. FIG. 1. *Parasitic female*. FIG. 2. *Vulva of parasitic female*. FIG. 3. *Tail of parasitic female*.

ably reduced behind the anus and terminating in a fairly short finger-like tail, broadly rounded at the tip. The tail (Fig. 3) varies in length from 0.075 to 0.109 mm. The eggs are ellipsoidal, thin shelled, and from 46 to 58 μ long by 23 to 29 μ wide. They are not fully embryonated in the uterus, but may contain an embryo at the time of elimination with the faeces. The uteri usually contain several fully developed ova and as many as 25 undeveloped eggs may be seen.

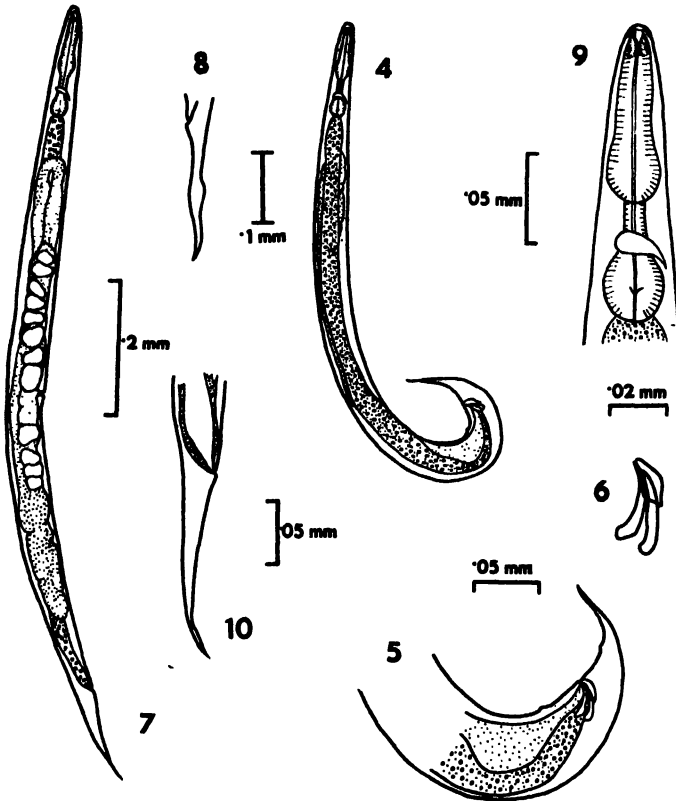
The Free-living Generation

This comprises male and female forms. The latter lay eggs that develop into rhabditiform larvae and subsequently into filariform larvae.

Male (Fig. 4). From 0.870 to 1.050 mm. in length with an average width of 0.058 mm. at the middle of the body, which is of more or less equal diameter except in the tail region. Anterior to the junction of the oesophagus and

intestine the head region tapers uniformly. Anterior to the anus the body shows marked thickening, but posterior to the opening it becomes considerably narrowed, tapering quickly and terminating in a fairly long slender point. The mouth leads into a short pharynx. The oesophagus is from 0.120 to 0.160 mm. long and is made up of two parts connected by a narrow neck. The anterior portion is more or less cylindrical with a slight swelling before narrowing to the neck region. A small area at the anterior end of the oesophagus shows a decided muscular thickening. The neck is fairly long and is followed by a round to flask-shaped, muscular bulb. The nerve ring crosses the neck just anterior to this bulb.

The male gonad is single and extends almost up to the oesophagus, occupying most of the width of the body. The terminal portion of the testis narrows considerably to form a duct leading into the cloacal opening. The tail (Fig. 5) is curved ventrally, and is from 0.090 to 0.165 mm. in length. Caudal papillae are present on the ventral surface though not very conspicuous—one pre-anal and one post-anal approximately equidistant from the anus. The spicules are 0.028 to 0.035 mm. in length and resemble a curved blade



FIGS. 4-10. FIG. 4. Free-living male. FIG. 5. Tail of free-living male. FIG. 6. Accessory piece and spicules. FIG. 7. Free-living female. FIG. 8. Abnormal tail of free-living female. FIG. 9. Anterior end of free-living female. FIG. 10. Tail of free-living female.

with a knob-like handle (Fig. 6). The accessory piece (Fig. 6) is from 0.018 to 0.023 mm. in length and is of an irregular oval shape.

Female (Fig. 7). From 0.975 to 1.350 mm. in length with an average width of 0.061 mm. immediately anterior to the vulva. Anteriorly the body tapers gradually; posteriorly the narrowing is slightly more abrupt, becoming considerably narrowed in the region of the rectum and tapering finally to a slender pointed tail. The tail was not observed to taper smoothly to a point in all cases; bulbar enlargements (Fig. 8) were observed frequently between the anus and tail tip. The head region and oesophagus of the female (Fig. 9) closely resemble those of the male. The oesophagus is from 0.140 to 0.188 mm. in length. The vulva shows well defined lips and is located slightly posterior to the middle of the body; the gonad is double and consists of anterior and posterior loops of the simple hair-pin bend type. The number of eggs present in the uteri is variable and as many as 23 were observed in well grown females. These ova are more or less sub-spherical in shape with a length of 38 to 62 μ , and a width of 28 to 40 μ . It has been observed that the female has not necessarily finished growing when eggs appear in the uterus. After oviposition is completed the uteri shrink considerably in size. The tail is slender (Fig. 10), pointed, and from 0.090 to 0.165 mm. long.

Rhabditiform larva

The rhabditiform larvae (Fig. 11) show considerable size range, depending on the stage of growth. At the time when the genital primordium is conspicuous, the larvae are approximately 0.4 mm. in length, with a width of 0.02 mm. The genital primordium is located in the region of the middle of the intestine. The tail is slender and tapering.

Filariform larva

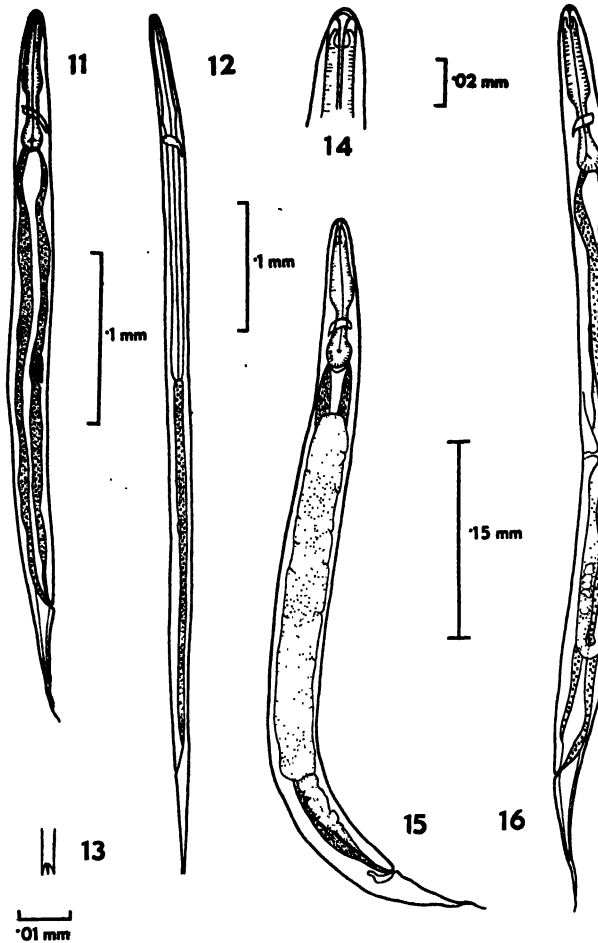
These larvae (Fig. 12) are from 0.645 to 0.760 mm. long and from 0.018 to 0.022 mm. wide in the region of the base of the oesophagus. The oesophagus is from 0.276 to 0.354 mm. long and shows a slight enlargement at the end of its first third; it joins the intestine just anterior to the middle of the body. The genital primordium is located at approximately the posterior end of the first third of the intestine. The tail is straight and tapered, showing a tricuspid termination (Fig. 13).

HOST: *Dasyprocta agouti*.

LOCATION: Small intestine.

TYPE LOCALITY: Trinidad, B.W.I.

The *Strongyloides* from the agouti differs from most of the other species on the basis of size. In this respect it falls between *S. papillosus* and *S. westeri*, though it is never as large as the latter. The majority of specimens are considerably larger than those of *S. papillosus* and the minimum lengths of oesophagus and tail are always greater than the maximum for that species. Moreover, in cross-infection experiments the agouti species will establish



FIGS. 11–16. FIG. 11. *Rhabditiform* larva (20 hr. old). FIG. 12. *Filariform* larva. FIG. 13. *Tricuspid* tail of *filariform* larva. FIG. 14. Head of *immature free-living female*. FIG. 15. *Immature free-living male*. FIG. 16. *Immature free-living female*.

itself readily in the guinea pig but not in the rabbit, whereas this condition is reversed with *S. papillosus*.

Comparative size, shape of tail, as well as the expulsion of embryonated ova in the faeces differentiated this species from *S. stercoralis*. No characteristic spear apparatus was seen in the oesophagus as found in the free-living female of *S. simiae*, and the marked post-vulvar constriction in the free-living females of *S. fülleborni* was not observed. The dimensions of the agouti species distinguish it from the much smaller *S. ratti*.

The twisting of the anterior and posterior ovarian loops does not show any constancy; and the shape of the tail of the parasitic female, although little variation is observed, cannot be considered as a distinctive characteristic.

This species, accordingly, differs from those that have been adequately described and is, therefore, referred to a new species under the name of *Strongyloides agoutii* sp. nov.

Recent Aspects on the Biology of *Strongyloides*

The parasitic generation in the genus *Strongyloides* is represented by females, considered by most authors to be parthenogenetic or hermaphroditic. These females lay eggs that may hatch within the intestine, so that free larvae are found in freshly passed faeces. In some species, however, the eggs themselves appear in the faeces. The larvae that are discharged in the faeces or hatched from the ova are known as rhabditiform larvae. These may metamorphose directly to the filariform or infective larvae or they may develop into a free-living bisexual generation of rhabditiform males and females. The free-living sexual generation produce fertile ova which give rise to rhabditiform larvae and later to infective filariform larvae. The former metamorphosis is known as the direct or homogonic type of development, the latter as the indirect or heterogonic method.

There are thus two possible cycles in the life history of members of this genus which permit alternative modes of origin of the infective filariform larvae. Since the occurrence of these two modes of development was confirmed, many hypotheses and theories concerning the factors influencing them have been offered.

Earlier workers considered that physical, chemical, and mechanical factors in association with environmental conditions, might influence the mode of development, but investigation did not confirm these views.

Recent workers, by the use of cultures in which both types had appeared, have been unable to produce changes in either type by subjection to controlled environmental conditions. The hypothesis that the direct developing type was confined to the temperate zone and the indirect to the tropics was accepted for some time (24, 25). Further suggestions were made that the differences in mode of development might be attributed to nutritional conditions to which the larvae are subjected prior to leaving the intestine of the host (Darling, 1911).

More critical methods of procedure and experimentation have proved the former theories to be untenable. Pure strains of one type were seldom observed and the general instability of types was accepted. The infection of certain abnormal hosts was found to lead to changes in the type of life cycle. Cytological studies of the gonads of the parasitic female led to the belief that the female is really hermaphroditic rather than parthenogenetic (28-31). This observation gave rise to a theory that the direction of development is determined by the chromosome constitution of the eggs after fertilization, and that the sex of the rhabditiform generation is determined according to whether the eggs are fertilized by sperm bearing, or not bearing, a heterochromosome (32).

The classical life cycle of the genus *Strongyloides* was generally accepted until comparatively recent years. In addition to the direct and indirect phases of development of *Strongyloides*, a distinct hyper-infective type was stated to occur, resulting from the rhabditiform larvae metamorphosing to

infective larvae prior to feeding (6, 7). This type is, therefore, the form responsible for the so-called "auto-infection" or "hyper-infection" of individuals that have become parasitized previously. At the same time, the administration of gentian violet as a strongyloidicide was believed to change the indirect and hyperinfective strains of *Strongyloides* to the direct type, a factor of no little importance to the fundamental biological ideas of the life cycle of the genus (8).

The discovery of male parasitic stages gave rise to further hypotheses concerning the mode of development of the free-living generation (9, 23). Evidence has been presented in support of the view that spermatozoa observed in the reproductive tubule of the female are the result of a process of insemination by a male. It is suggested that this process occurs during the latter part of the adolescent stage of the female, and that the insemination may take place in the lungs (where females and males have been observed together) prior to the migration of the female into the mucosa of the intestinal wall. This insemination is considered to suffice for several months after the females invade these tissues. The belief that fertilized ova give rise to an indirect type, while unfertilized ova may produce a direct type of development, was put forward. This idea is consistent with the tendency of strains that have dwelt within a host for any length of time to change from indirect to direct type as the supply of spermatozoa becomes depleted.

Experimental evidence has been presented to show the continued propagation of the free-living generation on artificial media (1, 2). It has been stated that when a known species of the genus, which most nearly demonstrates a typical indirect mode of development, is cultured under optimum conditions, it will continue to develop indirectly. However, when subjected to unfavourable environmental conditions (e.g., reduction in quantity or quality of nutrient or reduction in viscosity) a modification of the strain toward a direct type may be observed. Free-living adults of the first, second, and third generations were grown on artificial media; and it is believed that continued propagation of the free-living sexual generation occurs normally in nature under optimum conditions. This evidence would tend to suggest that "directness" and "indirectness" are conditioned by environment and not by the "genetical make up" of the ovum.

The biological studies on the genus during the past few years have tended to complicate rather than simplify the picture. As a result of critical investigations involving the transfer of a pure line strain of *S. ratti* through 14 parasitic generations by means of single larva transfers, it has been demonstrated that a male parasite is unnecessary in the bionomics of *S. ratti* either to maintain parasitic fertility or to explain the two modes of larval development (16-18). The theory of hyper-infection as a possible source of either hypothetical parasitic males or additional parasitic females is not supported, and the parasitic phase of *S. ratti* is considered to be represented by a female organism either syngonic or parthenogenetic.

It has recently been shown that the frequency with which the daily yield of offspring (resulting from singly established parasites of *S. rattii* in the rat) included adults of heterogonic development was subject to fluctuations over long seasonal cycles. Data suggest that the rat responds physiologically to changing meteorological conditions and that the altered environment thus produced for *S. rattii* leads to changes in the relative frequency with which progeny of heterogonic development are produced. All evidence from these recent studies indicates that the mode of larval development shown by the progeny of *S. rattii* is determined prior to oviposition (19).

THE FREE-LIVING OR SEXUAL GENERATION OF *Strongyloides agoutii*

It was considered advisable to continue observations of development of the sexual generation over as long a period as possible, in order to record any appearance of latent factors that might not reveal themselves in a short time. The observations extended from April 1936 until March 1939, some 139 cultures being made. The collection of at least three faecal samples per month seemed desirable, and as a general rule these were collected on different days; on occasions when cultures failed to furnish a sufficient nematode population, further samples were collected during that month. In this way, it was considered that any seasonal variation in development would be observed.

All cultures during 1936 were kept at room temperature, which ranged from 15 to 30° C., with a usual temperature of 23° C. During the first six months of 1937 the temperature was held regularly at 22° C., though on 15 occasions in May and June it ranged between 25 and 30° C. During the latter part of 1937 and throughout 1938 and 1939 the temperature variation was similar to 1936.

The routine procedure adopted in examination was modified to suit each set of cultures. However, since with this species ova are deposited in the faeces, the first examination of all cultures was made on the morning following the day of collection, usually 20 hr. after passage; subsequent examinations were daily or oftener, as necessary. If only filariform larvae were present on two or more consecutive examinations, the daily observations were discontinued and cultures were examined on alternate or on every third day. As the Petri plate lids were not lined with filter paper, it was usually necessary to moisten cultures at least once a week. Sufficient aeration was provided by removal of the lids when examination was carried out under the binocular microscope. In most instances, developmental forms could be differentiated under 16 to 32 magnifications, but if doubtful they were examined under the compound microscope. Cultures were discarded when two or more consecutive examinations showed no activity.

It will be observed that the total number of cultures recorded on the graphs does not correspond to the total examined in the investigation. A few records have been deleted when the culture has been rendered inactive soon after

origin, because of abnormal conditions; in some, larval development was not observed; in a few, all the larvae were used for experimental infection purposes. During the three-year period, time lost by sickness or holidays was responsible for the discard of some cultures. The analysis of these cultures shows the indirect or heterogonic type of development was consistent over the three years, showing no variation from month to month.

As a general rule, rhabditiform larvae were not observed on first examination of the faeces. In the majority larvae were not observed in any number for 48 hr., after which immature males and females were observed. In all instances females predominated in numbers, males being present in most but always in a great minority. The length of life of the male was considerably shorter than that of the female, the former dying after the second or third day of activity.

From Fig. 17 it will be seen that most free-living sexual forms appeared between the second and fifth days of culture and disappeared over a period of from 3 to 11 days. Free-living sexual stages disappeared from most cultures by the seventh or eighth day, although in four cases they lived as long as 11 days.

In Fig. 18 the first appearance of filariform larvae is indicated. In many cultures filariform larvae appeared on the fourth day, though in the greatest number they appeared on the sixth day; by the eighth day, larvae were present in all cultures.

The criterion of life of cultures is the activity of filariform larvae. From Fig. 19 it is seen that in the greatest number of cultures larvae became inactive between the 21st and 25th days. They remained active in some for 35 to 40 days, and in one only for 41 days. From Fig. 18 it is seen that filariform larvae appeared in the majority by the sixth day. Accordingly, the maximum duration of life of the filariform larvae is 35 days.

THE CONTINUED PROPAGATION OF THE FREE-LIVING GENERATION OF *Strongyloides agoutii*

The first intensive study of this nature with a member of the genus *Strongyloides* was made by Beach (1, 2). With both human and primate species of *Strongyloides*, he recorded continued propagation of the free-living phase of *S. simiae* on artificial culture media for three generations. His extensive investigations show that *S. simiae*, which follows the totally indirect type of development, changed to a mixed type when cultured on various artificial media. Parthenogenesis in the free-living female of this species was not found to occur. A single parasitic female, placed in half-strength Locke's solution, laid eggs that developed into males and direct filariform larvae in three cultures, while males, females, and filariform larvae developed in another.

Observations on the filariform larvae of *S. fülleborni* in different media as well as in tissue culture were recorded by Chung (5). In most instances there was no development, though he found that larvae survived for many

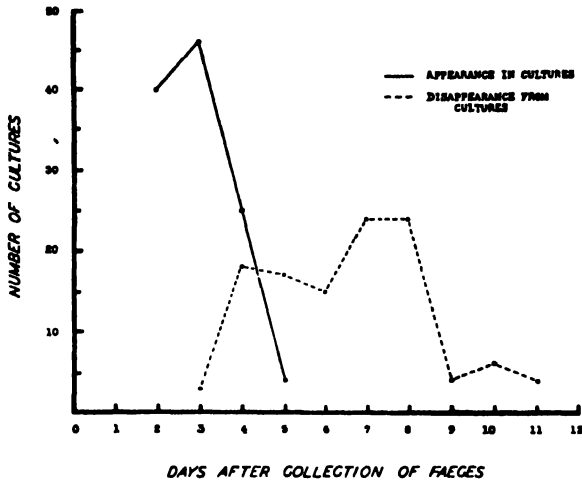


FIG. 17. Appearance and disappearance of free-living sexual stages of *S. agoutii* in faecal cultures from agouti.

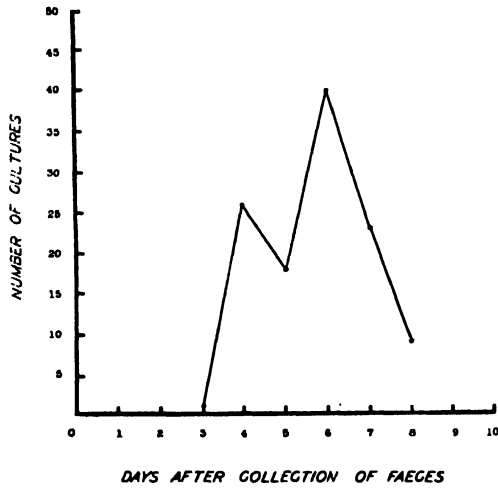


FIG. 18. Appearance of filariform larvae of *S. agoutii* after passage of faeces by agouti.

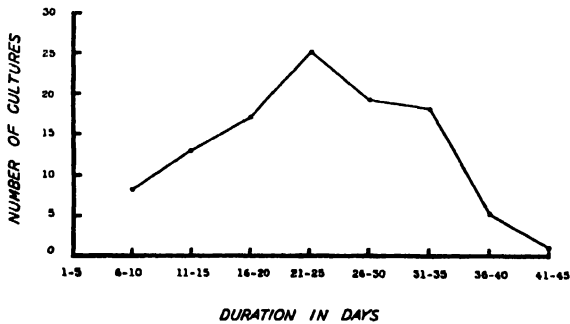


FIG. 19. Longevity of filariform larvae of *S. agoutii* in faecal cultures from agouti.

days in a medium having a pH between 5.4 and 7.0; a pH above 7.8 was unfavourable. Kouri, Basneuvo, and Arenas (22) report that with *S. stercoralis*, after numerous free-living generations, a change to an entirely free-living individual takes place, the female becoming parthenogenetic and there being no males. The fecundity of these females gradually decreases until the cultures become sterile. The findings of Beach (2) indicate that directness and indirectness in development of the free-living phase of the life cycle is contingent on environmental factors only. He considers that optimum conditions tend to produce the indirect type of development, whereas unfavourable conditions produce the direct.

In order to ascertain if continuous propagation of *S. agoutii* could be induced in artificial culture, a series of experiments was made.

To permit detailed observation of isolated forms in culture, a glass culture slide with a well 16 mm. in diameter by 3 mm. deep was employed. Small Petri dishes were also found satisfactory when it was desired to culture several individuals together. The medium used was similar to that used by Beach, with substitution of extract of agouti faeces in place of monkey faeces, and 1 gm. instead of 2 gm. of agar. It consisted of the following: 1 gm. of nutrient agar, 25 cc. of aqueous extract of agouti faeces, 75 cc. of distilled water. It was sterilized at 15 lb. for 20 min. to ensure the killing of any ova or larvae that might have been present. A thin film was placed on the bottom of the Petri dishes or of the well in the slides. This thin layer permitted the use of the compound microscope for examination purposes.

Larvae were obtained by Baermanning cultures of agouti faeces. Mature males and females were then selected. No attempt was made to control bacterial development in the cultures, but colonies were observed in only a few instances. It was necessary to moisten cultures quite frequently even though the slides were kept over water in large glass culture dishes, the lids of which were lined with filter paper to avoid excessive evaporation. Cultures were examined daily. Some 30 isolation cultures were made and examined in sets of six. The first group was cultured at room temperature of approximately 23° C. Slides No. 1, 2, and 5 were allotted two mature females each; No. 3, one immature female; No. 4, one female and one male; and two males to No. 6. With the exception of No. 1 and 4, the free-living stages were all dead by the fourth day; No. 1 and 4 showed active filariform larvae until the 18th and 10th days respectively.

Group 2 was cultured at 30° C. Slides were prepared with the following combinations: No. 7 with one male; No. 8 and 9 with a female each; No. 10 with a female and a male; and No. 11 and 12 with two females each. All individuals in this group were dead by the fourth day and no progeny was recorded.

Group 3 was cultured at room temperature. Slides No. 13-15 were made up with one female and one male each; No. 16 and 17 with two females each; No. 18 with two males. The males on No. 18 were dead by the third day.

Filariform larvae were observed in the other cultures and the duration of activity noted was 5, 6, 7, and 18 days.

Group 4 was cultured at 25° C. Slides No. 19-22 were allotted one male and one female each. No. 19 showed active filariform larvae up until the 11th day; No. 21 showed larvae until the fifth day; No. 20 and 22 produced no progeny. Progeny of No. 23, which comprised two females, were active until the 18th day; and the two males of No. 24 lived but three days.

Small Petri plates were used for cultures No. 25 to 30. The first three contained five females and one male each, the others, five females and five males each. The cultures were kept at room temperature (23° C.) and filariform larvae developed, but all activity ceased on the seventh and eighth days.

Discussion

A morphological and biological study of a hitherto unrecorded member of the genus *Strongyloides* has justified the creation of a new species, *S. agoutii*.

Variation in rate of growth and maturity of free-living forms was observed to occur, the regulating factor probably being food supply or environmental conditions of the media. The wide range of results, presented graphically, was unavoidable both on account of the actual error in observation and the excessive rate of metamorphosis of forms from one stage to another.

The frequency of the first appearance of filariform larvae on the fourth day might possibly be interpreted as the appearance of direct development in cultures. This occurrence could conceivably have been overlooked or not differentiated by the technical methods employed in routine observations. However, since the direct type of development was never observed, it seems improbable that it occurred.

From the data obtained, there is no evidence of continued propagation of the free-living generation of *S. agoutii*.

Acknowledgment

This investigation was undertaken at the Institute of Parasitology, Macdonald College, under the supervision of the Director, Prof. T. W. M. Cameron, who brought the agouti from Trinidad. The author wishes to express his appreciation and thanks to Professor Cameron for his continued interest, guidance, and generous assistance at all times throughout this study.

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CANADIAN WILTSHIRE BACON

V. QUANTITATIVE BACTERIOLOGICAL STUDIES ON CURING PICKLES¹

By N. E. GIBBONS²

Abstract

Five combinations of diluent, salt concentration of medium, and incubation temperature have been used to study the bacterial content of curing pickles used for making Wiltshire bacon. On representative pickles from 16 plants the highest mean count was on 10% salt agar (brine dilution) incubated at 20° C. The lowest count was on nutrient agar incubated at 37° C. Counts on media containing no salt, 4% salt, and 10% salt (water dilution) with incubation at 20° C. gave intermediate values. Counts on spent pickles were higher than on cover pickles. Pump pickles showed a surprisingly high number of organisms.

The analytical error attributable to diluting, plating, and counting was relatively small compared with the other sources of variance. Under certain conditions the error of sub-sampling a small jar of pickle exceeds the error between replicate plates. Sampling and sub-sampling errors were therefore the primary factors limiting the precision of the determinations.

Statistical analysis of the results showed that the differences in numbers observed by the different methods were highly significant for all pickles from 16 plants, and that the bacterial content of the pickles from the different plants differed significantly over all media. The number of bacteria observed by the different methods was usually correlated, i.e., pickles from a plant showing a high count by one method usually showed a high count by the other methods and vice versa. Nevertheless, it was possible to demonstrate a significant differential response of the bacteria in cover and spent pickles to the different growth conditions used. This suggests qualitative differences in the flora of the pickles. Of the several growth conditions tested, nutrient agar at 20° C. and 10% salt agar with brine dilutions appear to be the most suitable for demonstrating differential responses attributable to qualitative differences between the flora of different pickles.

Introduction

An outline of the investigation on Wiltshire-cured bacon has been given in a previous paper (2). The present paper deals with the quantitative bacteriological findings on representative curing pickles obtained from the various plants. Several combinations of growth conditions were used in order to obtain, in addition to the strict quantitative variations in bacterial populations, an estimate of the validity of this procedure for detecting qualitative differences in the bacterial flora of different plants.

Samples of the three pickles used for curing were obtained from each plant. The pump pickle, injected into the sides prior to cure, is usually a freshly prepared brine which might reasonably be expected to contain few bacteria. The tank pickle, used to cover the sides during cure, was analysed at the beginning and end of cure, these samples being designated "cover" and "spent" pickles respectively. Details of the design of the experiment and methods of

¹ Manuscript received February 9, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 38 of the Canadian Committee on Storage and Transport of Food. N.R.C. No. 905.

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sampling and of shipping the pickle have been described previously (2). Most pickles were sampled within an hour or two of receipt, although occasionally it was necessary to hold them as long as 12 hr. Pump pickles from 19 plants, and cover and spent pickles from 16 plants, furnished the data on which this paper is based.

Statistical methods were used to reduce and assist in the interpretation of the data (6). Since the bacterial numbers showed great variation, both between plants and the various cultural methods employed, the logarithms of the numbers were used both for convenience and valid interpretation on a statistical basis (1).

Cultural Methods

Lochhead (5) has shown that the count obtained on nutrient agar with incubation at 37° C., as commonly used in most packing plants for control purposes, is lower than that on nutrient agar or salt agar incubated at 20° C. Nutrient agar containing 10% salt, following dilution in brine of the same concentration, gave the highest counts, these being about 80 times greater than those obtained on nutrient agar incubated at 37° C. Water dilutions were found to render up to 90% of the organisms in pickle incapable of growth.

TABLE I
METHODS USED TO ESTIMATE THE NUMBER OF BACTERIA IN CURING PICKLES

Method	A	B	C	D	E
Temp. of incubation, °C.	37	20	20	20	20
Diluent	Dist. water	Dist. water	Dist. water	4% NaCl*	10% NaCl*
Medium	Beef-extract agar	Beef-extract agar	Beef-extract agar + 10% NaCl	Beef-extract agar + 4% NaCl	Beef-extract agar + 10% NaCl
Period of incubation, days	3	7	10	7	10

* % = Gm. added to 100 ml. of liquid.

These facts formed the basis for adopting the methods outlined in Table I. Method A was selected since it is commonly used for control purposes in packing plants. As a working hypothesis it was assumed that Method B would yield some estimate of the organisms introduced by the fresh sides; Method C, the salt-tolerating organisms capable of withstanding considerable change in osmotic pressure; Method D, the organisms capable of rapid growth on cured bacon; and Method E, the halophilic flora of the pickle. It is recognized that these methods may overlap considerably and that they do not yield an accurate estimate of the various types present. They were, however, considered suitable for a preliminary investigation of the subject.

Sampling

Since it was impossible to visit each of the plants, the samples were taken by a plant operator, who was provided with detailed instructions. By obtaining two samples of the pickle (taken at different times) from each plant,

it was possible to obtain an estimate of the combined error due to sampling and to any differential change that occurred in the samples during shipment. A discussion of this phase of sampling has been given (2).

Apart from any systematic change that may occur in the bacterial numbers during shipment at different temperatures over varying periods of time, it is of interest to point out that a sub-sampling error arising from the removal of duplicate samples from a small jar of pickle was greater in some instances than the analytical error of plating and counting. The results of typical experiments appear in Table II. It can be seen that the sub-sampling error is significantly greater than the analytical error in two experiments at 22° C. and approaches significance in the third. Of the six experiments at 1.1° C., three of which were shaken more frequently during storage than the others, in only two was the sub-sampling error significantly greater. In both instances the variance necessary for significance was contributed by one set of duplicates. Since this error is apparently greater when pickle is stored at the higher temperature, it is possible that it is the result of protein precipitation which interferes with the uniform distribution of the organisms through the sample.

TABLE II

SAMPLING AND ANALYTICAL ERRORS OF COUNTS ON PICKLES STORED UNDER DIFFERENT CONDITIONS

Pickle	Method	Storage temperature, °C.	Sampling and analytical error		Analytical error		F
			D.f.	Mean sq.	D.f.	Mean sq.	
1	B	22	7	.0817	14	.0062	13.18**
		1.1	6	.0178	12	.0083	2.14
		1.1	6	.0148	12	.0099	1.49
2	B	(shaken)					
		22	7	.0249	13	.0094	2.65
		1.1	6	.0021	12	.0041	0.51
		1.1	6	.0047	12	.0012	3.77*
	E	(shaken)					
		22	7	.0378	28	.0044	8.59**
		1.1	6	.0086	24	.0027	3.18*
		1.1	6	.0091	24	.0177	0.51
		(shaken)					

* Indicates 5%, ** 1% level of significance.

Quantitative Results

Typical arithmetic counts of cover pickles by the various methods are shown in Table III. The logarithms of the mean, maximum, and minimum number of bacteria per ml. over all plants are given in Table IV. In general the counts were maximal on 10% salt agar (Method E) and decreased with decreasing salt concentration (Methods D and B) and with increasing temperature (Method A). Dilution with water and incubation on 10% salt medium (Method C) gave results comparable with Method B. The mean

TABLE III
REPRESENTATIVE BACTERIAL COUNTS ON COVER PICKLES UNDER DIFFERENT CULTURAL
CONDITIONS. NUMBER OF ORGANISMS PER ML.

Sample	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	Dist. water 10% salt agar 20° C. (C)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)
1	700	5,000	7,400	60,000	900,000
2	75,000	96,000	57,000	300,000	470,000
3	12,000	36,000	19,000	61,000	120,000
4	46,000	150,000	170,000	900,000	4,700,000
5	70	600	500	770	600
6	630	12,000	11,000	30,000	34,000
7	39,000	130,000	63,000	500,000	490,000

TABLE IV
BACTERIAL COUNTS OF CURING PICKLES UNDER DIFFERENT CONDITIONS OF GROWTH

Logarithm of number of organisms per ml.

	Dist. water nut. agar 37° C. (A)	Dist. water nut. agar 20° C. (B)	4% brine 4% salt agar 20° C. (D)	10% brine 10% salt agar 20° C. (E)	Dist. water 10% salt agar 20° C. (C)
Pump pickle					
Mean	3.01	3.69	4.25	4.40	3.78
Maximum	4.65	4.89	5.40	5.98	4.89
Minimum	1.68	2.40	2.43	2.15	2.13
Stand. dev.	.71	.65	.88	1.10	.78
Coeff. of variation	23.6	17.6	20.7	25.0	20.6
Cover pickle					
Mean	3.63	4.36	4.96	5.33	4.29
Maximum	5.00	5.36	6.24	7.12	5.48
Minimum	1.14	2.11	2.23	2.46	2.21
Stand. dev.	1.09	.87	1.07	1.23	.87
Coeff. of variation	30.0	20.0	21.6	23.1	20.3
Spent pickle					
Mean	4.44	4.99	5.68	5.95	4.88
Maximum	5.22	5.78	6.89	7.15	5.87
Minimum	3.26	3.85	4.35	4.47	3.68
Stand. dev.	.51	.44	.56	.66	.51
Coeff. of variation	11.5	8.8	9.9	11.1	10.4

number of bacteria in cover and spent pickles from all plants shows that Methods A, B, and D measure about 2.5, 10, and 50%, respectively, of the number observed by Method E. This agrees quite well with Lochhead's figures of 1.3, 5.7, and 52.3% obtained from a number of observations on the curing pickle of one plant (5).

On all media the mean count increases in the order, pump, cover, and spent pickle. It is interesting to note that the freshly prepared pump pickle contains, on the average, almost as many organisms as the cover pickle. In fact,

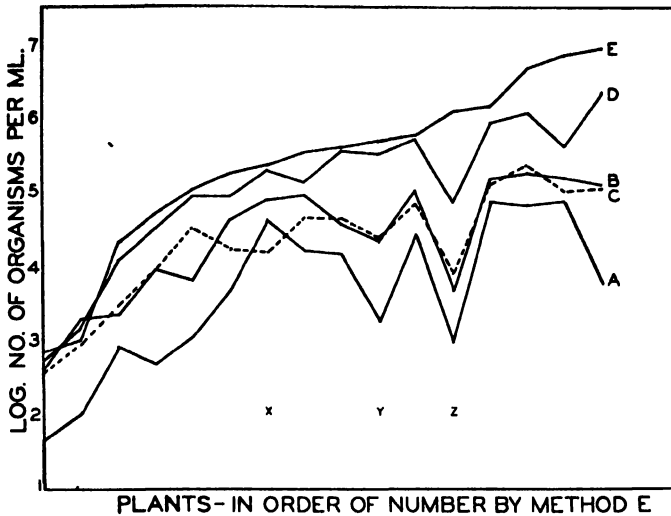


FIG. 1. Bacterial content of cover pickle from 16 plants as indicated by different cultural methods: A—nutrient agar incubated at 37° C.; B—nutrient agar incubated at 20° C.; C—10% salt agar from water dilutions; D—4% salt agar; and E—10% salt agar from brine dilutions. All incubated at 20° C.

the minimum values for pump pickle are usually higher than those for cover pickle.

The maximum and minimum values, the standard deviation and the coefficient of variation (Table IV) give some indication of the variation that occurs between the various counts and pickles. Since the standard deviation usually increases with the mean number present by each method, the coefficient of variation is relatively constant for each type of pickle. The standard deviation and coefficient of variation indicate that counts on nutrient agar at 20° C. are the least variable, both absolutely and relatively, while the 10% salt agar count is relatively the most variable for pump pickle and the 37° C. count relatively most variable for cover and spent pickles. It is evident that the spent pickle is the least variable of the three types. It would seem that the number of bacteria present in cover pickle reflects the many variations in pickle formula and plant practice, but during cure becomes more uniform.

Sources of Variability

The variability indicated by the standard deviation in Table IV can be divided into that originating from three main sources: (i) combined error of dilution, plating, and counting (analytical error); (ii) combined error of sampling the tank, differential changes during shipment, and sub-sampling the jar of pickle (sampling error); and (iii) the difference in count of pickles from different plants. Since the number of organisms in the pickle may vary from time to time in the same plant, the sampling error is probably over-estimated.

The analysis of variance of the counts on the three types of pickle is shown in Table V. In all cases the sampling error was significantly greater than

TABLE V

ANALYSIS OF VARIANCE OF BACTERIAL COUNTS ON PICKLES

Variance	Dist. water nut. agar 37° C. (A)		Dist. water nut. agar 20° C. (B)		4% brine 4% salt agar 20° C. (D)		10% brine 10% salt agar 20° C. (E)		Dist. water 10% salt agar 20° C. (C)	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump pickle										
Analytical error	71	.0042	72	.0014	74	.0020	72	.0023	74	.0020
Sampling error	19	1.1564**	19	.4593**	19	.3645**	19	.5663**	19	.5337**
Between plants	18	1.8093	18	2.0360**	18	4.3358**	18	6.7464**	18	3.1623**
Cover pickle										
Analytical error	64	.0142	61	.0022	62	.0012	61	.0026	61	.0034
Sampling error	16	1.1579**	16	.6007**	16	.2541**	16	.8702**	16	.8003**
Between plants	15	6.3017**	15	4.0321**	15	6.0120**	15	8.2778**	15	3.7652**
Spent pickle										
Analytical error	59	.0019	62	.0028	61	.0049	61	.0056	61	.0016
Sampling error	16	.3377**	16	.2597**	16	.2468**	16	.2741**	16	.2958**
Between plants	15	1.2070**	15	.9020**	15	1.6128**	15	2.3336**	15	1.2660**

** Indicates 1% level of significance.

the analytical error. Most of the variance attributable to sampling was contributed by a few plants showing considerable differences between the two samples. In spite of the large sampling error the difference between the bacterial numbers present in the pickles from different plants was highly significant in all but one instance. It can therefore be said with assurance that the bacterial numbers present in the pickles vary much more between plants than within plants, since the latter source of variance is included in the sampling error.

Relations of Counts by Various Methods

The numerical differences observed by the various methods may result from (i) essentially similar floras in all pickles with a fixed proportion of the maximum observed population developing under the different conditions, or (ii) different floras with a different fraction of the total population favoured by each method. Obviously a high degree of correlation between the quantitative counts by the several methods would indicate the first condition, while no correlation would suggest the second. Preliminary examination of the data indicated that there was some evidence favouring both of the above behaviours. For instance, a pickle having a high count by one method usually had high counts by all methods, suggesting a correlation between them. On the other hand, although the number of bacteria observed on nutrient agar incubated at 37° C. was on the average 2.5% of that observed on 10% salt agar incubated at 20° C., for the individual pickles this proportion varied from 0.08 to 16%. Although a general association exists between the bacterial numbers observed by the different methods for each cover pickle,

TABLE VI
CORRELATION BETWEEN NUMBER OF BACTERIA GROWING ON VARIOUS MEDIA

Quantities correlated		Correlation coefficient (<i>r</i>)		
		Pump pickle ¹	Cover pickle ²	Spent pickle ²
Number on nut. agar 20° C. <i>and</i>	Number on nut. agar 37° C.	.92	.94	.85
	4% salt agar	.89	.91	.90
	10% salt agar	.86	.86	.77
	10% salt agar (water)	.94†	.93	.92
Number on 4% salt agar <i>and</i>	Number on nut. agar 37° C.	.74†	.90	.81
	10% salt agar	.97†	.91	.81
	10% salt agar (water)	.94†	.96	.91
Number on 10% salt agar <i>and</i>	Number on nut. agar 37° C.	.72†	.81	.70
	10% salt agar (water)	.94†	.92	.83
Number on nut. agar 37° C. <i>and</i>	Number on 10% salt agar (water)	.83	.87	.80

¹ 17 degrees of freedom, *r* of .58 required for 1% level of significance.

² 14 degrees of freedom, *r* of .62 required for 1% level of significance.

† Significantly different from †.

as shown in Fig. 1, there are some wide fluctuations as indicated when the numbers obtained from the plants marked *y* and *z* are compared with those from plant *x*.

In order to place these observations on a more quantitative basis, correlation coefficients were computed between the numbers of bacteria observed by the different methods. The values obtained appear in Table VI. All of these coefficients are statistically significant, thereby demonstrating a definite association between the numbers of bacteria indicated by the different methods. In other words, a pickle having relatively large bacterial numbers by one method will generally have relatively large numbers by all methods. On the other hand, the magnitude of the correlation coefficients is such that the residual variance unaccounted for by the coefficient varies from 5 to 50% of the total variance. This shows that there is a considerable element of independent fluctuation between the numbers observed by the different methods. These independent fluctuations responsible for the residual variance may be attributable wholly, or in part, to experimental error, or, as suggested earlier, to the possibility that the various cultural methods are capable of distinguishing to some extent a real difference between the floras present in different pickles. In order to determine the significance of these observations it was necessary to determine the differential response of the bacterial population to different cultural methods in a manner which permitted comparison with the known experimental errors. This suggested a variance and regression analysis of the data.

The differential response of the flora in the different pickles to the different cultural conditions is included in the interaction mean square, plants \times methods (Table VII). This interaction should be tested for significance by comparing it with the variance between aliquots. Since duplicate determinations were not made on each medium this comparison was impossible. However, it has been shown (Table II) that for pickles kept at low temperatures the error between aliquots (sampling and analytical error) was seldom significantly greater than the analytical error alone, and in consequence the largest analytical error observed was used for comparison. On this basis the interaction mean square was found to be overwhelmingly significant.

TABLE VII

VARIANCE AND REGRESSION ANALYSES OF COUNTS BY ALL METHODS

Nut. agar 37° C.; nut. agar, 4% and 10% salt agar at 20° C.; 10% salt agar from distilled water at 20° C.

Variance	Pump		Cover		Spent	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Between plants	18	15.9807**	15	11.3190**	15	6.3109**
Between media	4	33.5442**	4	94.6674**	4	36.2393**
Differential plants \times media	72	.6703**	60	.7307**	60	.3137**
Greatest analytical error	71	.0042	64	.0142	61	.0056
Greatest sampling error	19	1.1564	16	1.1579	16	.3377
Regression						
Differences in regressions	18	12.2352**	15	8.4022**	15	3.5119**
Residuals	57	1.2185	48	2.8537	48	1.2554

** Indicates 1% level of significance.

The fact that this plants \times media interaction derived from an analysis of variance was statistically significant does not provide definite evidence that the organisms present in the different pickles respond differentially to the different growth conditions. The magnitude of the interaction may be affected by the number of organisms present in the pickle as well as by differential effects attributable to differences in flora. Since the number of organisms in the different pickles has been shown to differ significantly, it is necessary to determine the true differential effect from a regression analysis (7). It was then found (Table VII) that the differences between regressions accounted for the major portion of the interaction variance in pump pickle. There is, therefore, little evidence to indicate that different types of floras are present in this pickle. For cover and spent pickles the variance due to differences in regressions, although significant, accounts for only about half the interaction variance. The remainder is therefore attributed to differential response of the organisms to different growth conditions. This finding suggests some difference in the types of organisms present in the different pickles.

The various cultural methods included the effect of salt concentration, temperature of incubation and dilution with water and 10% brine. A further study was made to determine which of these factors contributed the most variance to the observed interaction (Table VII). The results, appearing in Table VIII, show that an increase in salt concentration from 0 to 10% in the medium contributes significantly more to the interaction than increasing the incubation temperature from 20° to 37° C. with a salt-free medium. The effect of dilution method (water or 10% brine) has a smaller differential effect than the salt concentration in the medium but a somewhat greater effect than the incubation temperature.

TABLE VIII

INTERACTIONS OF PAIRED METHODS SHOWING EFFECT OF TEMPERATURE OF INCUBATION, AND SALT CONTENT OF MEDIUM AND DILUENT

		Effect of					
		Temperature of incubation		Salt conc. of medium		Salt conc. of diluent	
		D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Pump	Interaction	18	.1722†	18	1.2458†	18	.6294†
	Greatest anal. error	71	.0042	72	.0023	72	.0023
Cover	Interaction	15	.4259†	15	1.2377†	15	.9579
	Greatest anal. error	64	.0142	61	.0026	61	.0034
Spent	Interaction	15	.1650†	15	.5304†	15	.9553†
	Greatest anal. error	62	.0028	61	.0056	61	.0056

All interactions significant to 1% level.

† Significantly different from ‡.

These results show that the bacteriological difference between corresponding pickles from different plants is mainly quantitative. Cover and spent pickles, however, also show some differential response when cultured by different methods, suggesting qualitative differences in the flora. Media containing no salt and 10% salt incubated at 20° C. appear to be the most effective for demonstrating this differential response.

These findings are in agreement with practical conclusions. Since pump pickle is made in all plants from similar ingredients, quantitative rather than qualitative differences might be expected between different plants. However, because of the many methods of reclaiming spent pickle and the varying sources of contamination for cover pickle some differences in the types of organisms found in these pickles in different plants are possible.

Correlation of Bacterial Count with Chemical Composition of Pickles

Since bacterial activity is considered responsible for some changes in pickle composition during cure, correlation coefficients were computed between the differences in the logarithms of the number of bacteria present in the cover and spent pickles, or the relative growth rate during cure, and several constituents of the pickle (3). The correlations between the growth rate observed

in 4 and 10% salt media incubated at 20° C. and the mean value of certain components of the pickles appear in Table IX. None of these correlations was statistically significant for the 15 degrees of freedom available. Nevertheless the coefficient between pH and the relative growth rate was high enough to suggest some relation, the growth rate increasing as the pH increases within the range of observed values (4). Computation of this correlation on the basis of the results from individual pickles did not yield significant values, although twice as many degrees of freedom were available. Correlations between the arithmetic increase in bacterial numbers during cure and the nitrate and nitrite contents and the pH of the pickles were also insignificant.

TABLE IX

CORRELATION BETWEEN BACTERIAL GROWTH RATE AND MEAN VALUE OF CHEMICAL CONSTITUENTS OF COVER AND SPENT PICKLES

Quantities correlated		Correlation coefficient (<i>r</i>)
Log difference of counts on spent and cover pickles (4% salt agar)	Salt	-.01
	and Nitrate	-.12
	Nitrite	.02
	pH	.45
	Protein	-.33
Log difference of counts on spent and cover pickles (10% salt agar)	Salt	.19
	and Nitrate	.22
	Nitrite	.11
	pH	.36
	Protein	-.01

15 degrees of freedom, *r* of 0.48 required for 5% level of significance.

Actually a correlation with salt content could hardly be expected. The salt content of pickle varies from about 22 to 28%, and for halophilic organisms variations in salt content at concentrations about 20% have little effect. On the other hand, organisms affected by salt concentration are usually inhibited by concentrations much below this. A correlation between the increase in the number of bacteria, or of nitrate reducing organisms, and the total nitrate reduced might be expected, but under plant conditions it is very difficult to differentiate between the nitrate reduced and that absorbed by the sides since the combined errors applicable to the several determinations are large compared with the quantity of nitrate reduced. Likewise a measure of the total nitrite formed is difficult since the amount that reacts with the muscle proteins is unknown. Until these total quantities can be determined correlations of chemical constituents and bacterial numbers of pickle seem unlikely.

Acknowledgments

In addition to the managements of the various plants and those acknowledged in the first paper of this series, the author is indebted to Drs. J. W. Hopkins and W. H. Cook for advice in making and interpreting the statistical analyses, and to Mr. A. Morrison for assistance in making the counts.

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CANADIAN WILTSHIRE BACON

VI. QUANTITATIVE BACTERIOLOGICAL STUDIES ON PRODUCT¹

By N. E. GIBBONS²

Abstract

Surface counts of bacteria on the ribs of bacon showed that nutrient agar containing 4% salt incubated at 20° C. gave the maximum number. In sampling the surface of a side of bacon, it was found that the removal of the surface layer of tissue yielded more accurate values than methods based on the removal of organisms by swabs or filter paper impressions.

On the average, the bacterial load on the anterior ribs was greater than on the posterior ribs. Although there were significant differences in the number of bacteria on sides from the same plant, the greatest variation was between sides from different plants.

A visible growth of bacteria or "slime" becomes evident on the average when the logarithm of the number of organisms per sq. cm. exceeds 7.2. Nevertheless, certain sides may appear slimy at log 6.7 per sq. cm., while others will not show this condition at log 8.0 per sq. cm. This variation in the number of organisms present at the visible slime level may result from differences in the flora, different types of growth of the same organism, or variability in the method of detection.

The number of bacteria on the side was found to be correlated with the age of the sides from cure or from packing. The growth rate is slow during the first 8 to 10 days from packing, after which it increases. Sides having an initial load of 100,000 organisms per sq. cm. at packing may be expected to remain free from slime for 20 to 25 days, if stored at 1.1° C. No correlation was obtained between the number of bacteria in the curing pickle and the number on the product.

Introduction

Wiltshire sides are matured for a week to ten days after removal from the curing tank. Whether bacteria play a part in this process is not known, but there is a development of organisms on the surface of the meat which eventually may lead to undesirable features, such as slime or taint.

This study was undertaken to determine the variation in the bacterial load on sides of factory-cured Canadian Wiltshire bacon at the time of export, and the effect of transport or storage on the increase in bacterial numbers and the appearance of slime. For this purpose two sides from each of 22 plants were examined. The treatment of these sides, both before and after reaching the laboratory, has been described elsewhere (2).

Cultural Methods

Some preliminary work was necessary to determine the best medium for growth and the best method of enumerating the bacteria on the surface of bacon. Many of the types found in pickle must be present on the sides when

¹ Manuscript received February 9, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 39 of the Canadian Committee on Storage and Transport of Food. N.R.C. No. 906.

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removed from cure. While conditions may not be favourable for the development of all types present, some are able to grow as shown by the production of slime.

It was found that bacon infusion agars yielded slightly higher counts than beef extract agars, but the difficulty in preparing a clear medium, and the fact that the infusions contained varying amounts of salt, precluded their use as a routine medium. Beef extract, peptone agars were therefore used throughout the investigation.

To determine the optimum salt concentration, samples taken from cured bacon were diluted in brine containing from 0 to 10% salt, cultured on beef extract agar containing the same concentration of salt, and incubated at 20° C. The highest counts were obtained in the range 3 to 6% salt, the numbers falling off rather sharply at higher and lower concentrations. Within this range the maximum usually occurred on 4 to 5% salt. Since there is some advantage in using the lowest salt concentration compatible with good growth, a concentration of 4% salt was chosen with dilution in brine of the same concentration. It is of interest to note that the salt concentration of the bacon is usually about 4 to 5% (3). Consequently, it is evident that the conditions on bacon favour the development of organisms of intermediate salt tolerance.

Occasionally, counts equal to, or even higher than, those on 4% salt were obtained on 10% salt agar. It is conceivable that organisms from pickle, which prefer or are capable of growing at higher concentrations, sometimes persist for a time on the product.

Sampling

Three methods of taking the samples for surface bacterial counts were studied. The first of these, described by Haines (7), consists of outlining a definite area with sterile borers, and then removing a thin layer of the surface meat, or, in the case of ribs, the pleural membrane. Stainless steel borers, cutting a 3 sq. cm. circle, were used in this work. The second method, described by Garrard and Lochhead (4), consists of pressing a piece of filter paper of known area against the surface of the meat for 20 to 30 sec. The third was a swab method, with a piece of sheet aluminium having a 1 × 2 cm. opening used as a mask. In practice the mask was placed against the meat and the exposed area swabbed vigorously with two pledgets of absorbent cotton. The pieces of excised tissue, filter paper, or cotton obtained were in each instance transferred to dilution blanks containing coarse silica sand and shaken vigorously 100 times.

These methods were compared by taking duplicate samples by each method from the 3rd, 5th, 7th, 9th, and 11th ribs of a side of bacon. The position on the rib, from which each of the individual samples was taken, was determined by preparing a random sampling chart. The results were subjected to an analysis of variance which appears in Table I. The mean counts by the three methods show that the numbers obtained by the Haines and swab

techniques are in close agreement on the average, while that by the filter paper procedure is significantly lower. The mean square between duplicates shows that the precision decreases in the order: filter paper impression, Haines, and swab techniques. However, on the small number of observations made, only the swab technique can be said to be significantly less precise than the other methods.

TABLE I
ANALYSIS OF VARIANCE OF COUNTS ON BACON BY THREE METHODS

Method	Log mean no./cm. ²	Variance between duplicates		Variance between ribs		F
		D.f.	Mean sq.	D.f.	Mean sq.	
Haines	5.41	5	.028	4	.086	3.07
Filter paper	4.98	5	.013	4	.240	18.46**
Swab	5.40	5	.138	4	.041	0.30

** Exceeds 1% level of significance.

The filter paper impression method alone showed a significant difference between the numbers of bacteria on different ribs. Since the other two sampling procedures yielded similar results and showed no differences between ribs, it is concluded that the filter paper method, although precise, gives a less accurate estimate of the actual number of bacteria present. Examination of the detailed results showed that on an arithmetical basis the filter paper method gave about 75% of the count obtained by the Haines method on the 3rd rib and only 15% on the 11th rib. Since a similar gradient was not observed by the other methods on this particular side, and as the rear ribs are usually drier than those nearest the shoulder, it appears that the filter paper method may be affected by moisture conditions on the surface.

This effect of moisture was also observed for cut surfaces of meat. Pieces of filter paper of sufficient size were used to permit a sample to be taken afterwards from the same area by the Haines technique. On somewhat dry surfaces only about 10% of the organisms were removed by the filter paper when compared with the total number removed by the combined methods. On moister surfaces, up to 30% of the organisms could be removed by the filter paper.

The criticism may be made that on meat surfaces the Haines method may also include organisms from the sub-surface layers. However, similar results were obtained on rib surfaces where only the pleural membrane is removed. Since this membrane is not disintegrated when shaken with sand, only surface organisms would be removed.

The swab method is the least precise of the three. It should be mentioned that only after considerable experience with it could the high counts reported be attained. The Haines technique was therefore adopted in this study. Although it has the disadvantage of disfiguring the sides, it gives the best estimate of the actual number of organisms present, and in practice is probably the simplest to use.

Routine Procedure

In the examination of the sides from the various plants all counts were made on the pleural membrane, since this surface has the least opportunity of outside contamination, and slime generally appears there first. Areas of 6 sq. cm. were removed from the pleura over the 2nd and 3rd ribs and over the 9th and 10th ribs, and 3 sq. cm. from the 5th rib. The 5th and 6th ribs were then removed without contamination of the pleura and stored at 1.1° C. in an atmosphere of about 95% relative humidity (over saturated zinc sulphate solution) for 15 to 17 days. The sides were rebled and stored at 1.1° C. for 10 to 12 days as previously described (2).

At the second sampling 6 sq. cm. samples were taken from all ribs. In all cases dilutions were made in 4% brine and cultured on 4% salt agar. Counts were made after 7 days' incubation at 20° C.

Quantitative Results

Statistical methods (8) have been used to interpret some of the data. Since there was a wide variation in the number of bacteria found, the numbers were converted to logarithms both for convenience and valid statistical analysis (1).

The logarithms of the mean, maximum, and minimum number of organisms per sq. cm. are shown in Table II. From the means it may be seen that there was a slight decrease in bacterial numbers on the pleura from the anterior to posterior ribs both before and after storage. At both samplings the counts for the anterior ribs showed the greatest variation. Since the 5th rib was stored for a longer period, the second sampling from it cannot be compared with the others.

TABLE II

BACTERIAL COUNT ON PLEURAL MEMBRANE OF WILTSHIRE SIDES

(Logarithm of number of organisms per sq. cm.)

	Ribs 2 and 3		Ribs 9 and 10		Rib 5	
	Received	Stored 10-12 days	Received	Stored 10-12 days	Received	Stored 15-17 days
Mean	4.54	6.03	4.21	5.18	4.51	6.94
Maximum	6.27	8.55	5.64	7.41	6.56	9.25
Minimum	2.97	3.48	2.90	3.19	3.42	4.21
Stand. deviation	.67	1.19	.55	.93	.60	1.16
Coeff. of variation	14.8	19.8	13.0	18.0	13.3	16.7

The maximum and minimum values, the standard deviation, and the coefficient of variation (Table II) give some indication of the variation that occurs between the different sides and also between ribs. Since the standard deviation decreases with the decrease in mean number present, the coefficient of variation is fairly constant.

Sources of Variability

The variability shown by the standard deviation in Table II may be divided into that arising from three main sources: (i) the error of diluting, plating, and counting; (ii) differences in the bacterial load of sides from the same plant; and (iii) differences in the product from different plants. The variance due to these three sources is shown in Table III. For all three positions on the side the variance due to differences between sides from different plants is significantly greater than that due to differences between sides from the same plant, which in turn is significantly greater than the experimental error. The greatest source of variability is therefore between plants.

Appearance of Slime

A side may be said to be slimy when the bacteria have increased sufficiently to be visible or tactile. Although not of serious practical importance unless very pronounced, slime is considered to be an indication of improper handling at some stage during the history of the product. If the numbers are sufficient to be discerned, it is probable that taint will soon be detected.

Many of the 5th and 6th ribs that had been stored at 95% relative humidity for 15 to 17 days showed slime, and furnished an opportunity of determining the number of organisms necessary for a visible growth. On arranging the values for slimy and non-slimy sides (Fig. 1), it was found that there was a region in which sides having the same count may or may not appear slimy. Pleural membranes having a count of 5,000,000 per sq. cm. (log 6.70) or less were never slimy; with a count of 93,000,000 per sq. cm. (log 7.97) or over, the pleura was always slimy. Between these limits there were nine slimy and nine non-slimy ribs having an average count of 16,500,000 organisms per sq. cm. (log 7.22 ± 0.093). It is therefore probable that any side having a count of 16 to 17 million organisms per sq. cm. will be slimy. The counts obtained on the sides stored for a shorter time confirmed these results (Fig. 2). Haines (6) has found that slime becomes visible on stored beef at a similar value (log 7.5).

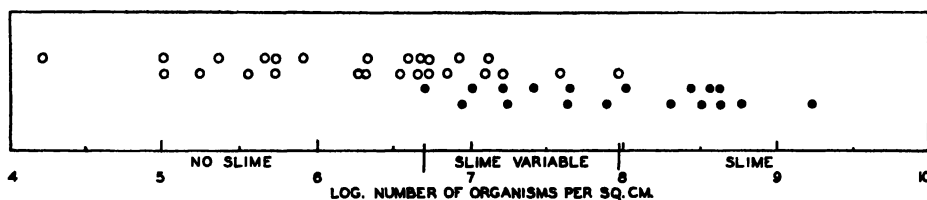


FIG. 1. *Relation between number of organisms on pleural membrane of Wiltshire sides and appearance of slime.*

In this study a visible growth was used as the criterion of slime. In practice a side may also be considered slimy if it feels slippery or if a growth can be detected by running the thumb nail over the surface. These subjective methods of detecting slime are doubtless liable to considerable variability,

TABLE III
ANALYSIS OF VARIANCE OF COUNTS ON BACON FROM 22 PLANTS

Variance	Ribs 2 and 3						Ribs 9 and 10						Rib 5			
	Received			Stored			Received			Stored			Received		Stored	
	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Analytical error	86	.0040	82	.0084	87	.0016	85	.0119	86	.0030	86	.0022	86	.0030	86	.0022
Between sides from same plant	22	.4671**	21	1.5617**	22	.2553**	22	1.0472**	22	.3641**	22	1.4774**	22	.3641**	22	1.4774**
Between sides from different plants	21	2.2882**	20	7.0647**	21	1.5696**	21	4.1791**	21	1.8034**	21	6.6714**	21	1.8034**	21	6.6714**
Differential change with time between plants	20	2.507**			21	1.816**			21						2.339**	
Average change with time over all plants	1	127.545**			1	63.445**			1						397.096**	

**Exceeds mean square error or intra-plant variance, respectively, 1% level of significance.

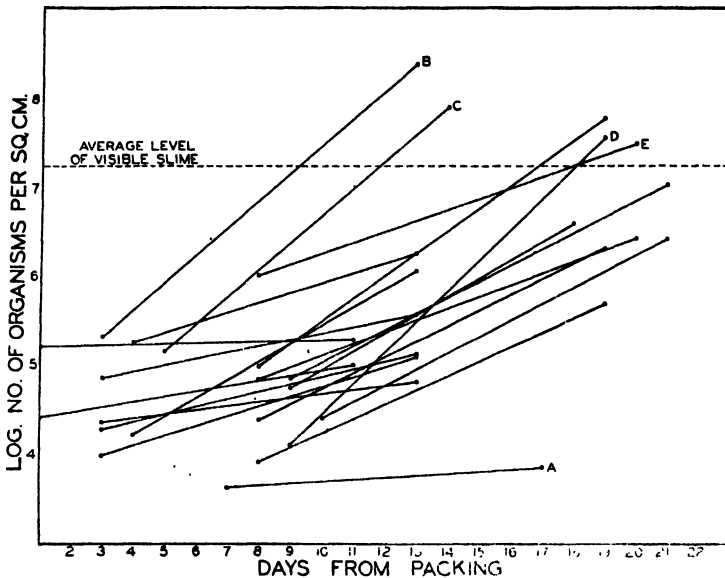


FIG. 2. Relation between number of organisms on pleural membrane of Wiltshire sides and age from packing. (Sides B, C, D, and E showed definite slime.)

even within a given method. Consequently, variations in the number of organisms present when a side is considered to have become slimy are to be expected. This may account for all or part of the observed variability. However, part of the variation may be real, since the visibility of equal numbers of organisms may be affected by the type of bacteria present, or by the conditions of growth on a particular side.

The significant interaction (differential change with time between plants, Table III) indicated that the bacteria do not increase at the same rate on sides from different plants. This is apparent from the slopes of the lines in Fig. 2 and suggests that the flora of different plants varied. It would appear that the flora of the sides from plant A is quite different from that of sides from plant B. On the other hand, sides from plants B and C, and possibly D, seem to have a similar flora, which grows very rapidly and is soon apparent as slime. Reports on the conditions of sides arriving in England showed that the product from plant B had a higher percentage of slime than that from the others. The conclusion seems justified that, while the majority of plants apparently have very similar types of organisms present on the sides, in a few instances quite different types may be present.

Relation of Number of Bacteria Present to Time

A significant correlation coefficient indicated that there was a relation between the number of bacteria on the sides at successive samplings and the time from the end of cure to each sampling (Table IV). The bacterial count was also correlated with the number of days from the beginning of cure and from packing.

On plotting the data (Fig. 2), this relation was confirmed and another demonstrated. Since the beginning and end of each line in Fig. 2 represent the number of bacteria present at the first and second samplings respectively, the slope of the line indicates the growth rate during this time. It can be seen that, if plants such as A, B, and C are excluded, the growth rate on sides sampled for the first time 2 to 4 days from packing is much less than on sides first sampled 8 to 10 days from packing. Although the lines are based on only two determinations, it is probably safe to conclude that under the conditions of storage there is a period of about 10 to 12 days during which the bacteria develop very slowly on the sides. After this time, development is much more rapid.

Since the appearance of slime depends on the number of bacteria present, it is evident that all sides will eventually become slimy. Apart from those having "abnormal" floras, the original load is important in determining how long the side remains free of slime. Sides having a high initial load (plant E) become slimy before those having a low initial load. From the evidence presented it may be said that, in general, if a side has a count of 100,000 per sq. cm. (about log 5) or less when packed, it will remain free of slime for 20 to 25 days under the usual conditions of transport.

TABLE IV
CORRELATION BETWEEN NUMBER OF BACTERIA ON PRODUCT, NUMBER IN PICKLE,
AND TIME

Quantities correlated		Correlation coefficients (<i>r</i>)		
		Ribs 2 and 3	Rib 5	Ribs 9 and 10
Log of no. of organisms per sq. cm. on each side regardless of origin (total 40 sides)	Days to each sampling from end of cure	.60**	.80**	.56**
	and Days to each sampling from packing	.58**	.80**	.57**
Log mean no. of organisms per sq. cm. on 2 sides originating from each of 20 plants	Days to each sampling from end of cure	.64**		
	and Days to each sampling from packing	.61**		
	Days start of cure to each sampling	.63**		
Log mean no. of organisms per sq. cm. on 2 sides from each of 20 plants (1st sampling)	No. of bacteria per ml. in curing pickle at end of cure (4% salt agar)	.32†		
	(10% salt agar)	.25†		
	(Nut. agar 20° C.)	.28†		
	(Nut. agar 37° C.)	.14†		

** Exceeds 1% level of significance, 38 degrees of freedom, *r* of .42 necessary for 1% level of significance for 35 D.f.

† 15 degrees of freedom, *r* of .48 necessary for 5% level of significance.

Relation of Number of Bacteria on Product to Number in Curing Pickle

The organisms found on bacon may come from a number of sources. Garrard and Lochhead (4) point out that the contamination on the sides prior to cure probably plays an important role. It is also possible that the organisms in curing pickle may play a part and the variation found in the number of bacteria in curing pickles (5) might be reflected in the number found on the product. To test this assumption the correlation coefficients shown in Table IV were computed. The number of bacteria on the bacon was not significantly correlated with the number of bacteria in the pickle in which it had been cured. However, the bacon examined varied in its age from cure and had been subjected to the various wiping and packing practices, and shipping conditions followed in the different plants. These factors, among others, are probably more important than the number of organisms in the pickle in determining the number of bacteria on the cured bacon.

Acknowledgment

The author is indebted to Mr. A. Morrison for technical assistance throughout this study.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

JUNE, 1940

NUMBER 6

CANADIAN WILTSHIRE BACON

VII. SPECIFICATION OF COLOUR AND COLOUR STABILITY¹

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Abstract

A method of specifying the colour of bacon by measurement of its red, green, and blue spectral components is described.

Statistical evidence, based on a total of 792 observations, is presented which indicates that although the variation of these three components of colour from sample to sample of meat is to some extent correlated, each also exhibits a significant element of independent fluctuation. It is accordingly concluded that for investigational purposes, all three components should be included in forthcoming studies of colour and colour stability, and factors influencing them, in an extended series of samples. The possibility that for routine operations such as plant control a more limited analysis might suffice, will, however, also be made the subject of inquiry.

Introduction

Owing to the economic importance of the colour and colour stability of bacon exported from Canada, a study of these properties was included in a recent investigation (1) of some of the factors influencing Wiltshire bacon quality.

By visual inspection alone, it is possible to recognize considerable variation in the colour of different bacon samples, even when drawn from a single packing plant. In this case, the observed variation is probably due mainly to inherent differences in carcasses prior to curing. With product from different plants, the variability may be expected to be enhanced by differences in the curing practices employed. In these circumstances, it may be inferred that for an extensive study of the colour of bacon from various sources, a simple method of colour measurement, adaptable to routine procedure, is both adequate and desirable. A method by which only the red, green, and blue components of the colour are determined (5) was accordingly adopted.

Samples of meat may differ from one another in respect of either or both of two attributes of colour, namely chroma or colour quality, and total intensity or brightness. Variations in the former arise from differential reflection of one or more of the incident wave-bands by individual samples. Brightness,

¹ Manuscript received February 23, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 40 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 911.

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on the other hand, is determined by the ability of the meat to reflect all components present in the incident light. Actually, the analysis of samples with respect to colour quality is believed to be the more important, as hue rather than intensity of colour seems to be primarily responsible for the observed variation in the initial appearance of different sides, as well as for the grey or brown discolorations sometimes developing on exposure to air.

In this investigation the percentage reflection of each colour component, relative to that of a white reference standard, provided information respecting colour quality. Total reflection was not measured specifically, but the sum of the percentage reflections for the three components yielded some indication of brightness. Certain complications must however be recognized in the relation of this index of brightness to visual appearance. Most artificial light sources, including that used in this study, are much richer in the red component than in the green or blue. As bacon is likewise predominantly red, much more of the red than of either the green or blue components is reflected from the meat surface. This combination of low incidence of green and blue light with the low reflecting power of bacon for these colours necessitated the use of much more intense illumination in the measurement of the percentage reflection of these than was required in the case of the red component. In fact, in order to obtain comparable degrees of precision, each of the three colour bands was studied at a different intensity of white light. This does not affect the estimation of the percentage reflection of each component, which is independent of the incident intensity, but does suggest that the summation of the three percentage values on equal terms may exaggerate the effect of differences in the blue and green components on the visual appearance of samples, even in white light.

Experimental

The colour measurements to be considered in this connection were made on samples from 44 Wiltshire sides, comprising two sides from each of 22 Canadian packing plants. Three samplings were made of each side, by methods described in the introductory paper of this series (1). On each such occasion two observations, namely of initial colour at the time of sampling, and of colour stability as indicated by the changes after exposure for 20 hr. at 10° C. and 95% relative humidity, were made on each sample, also by procedures previously outlined (5). There were thus 792 observations in all.

Chroma or Colour Quality

In earlier publications (5, 6), colour quality was defined in terms of intensity ratios, namely, red : green, red : blue, and green : blue. Statistical analysis of this property is, however, more simply accomplished by a consideration of the variance and covariance of the component intensities themselves, rather than of their ratios, comparisons of the latter being complicated by the fact that the ratios secured are not independent of the general intensity

level. This procedure will accordingly be adopted in the subsequent papers of this series.

It was not known *a priori* whether the measurement of all three of these components was actually necessary in order to specify the colour and colour stability of bacon. This would be the case if all three components were to vary to some extent at least independently from sample to sample of meat. On the other hand, if the fluctuations in the three components, although not necessarily of the same absolute magnitude, are nevertheless closely correlated, measurement of one component alone would suffice to specify all of them. Alternatively, an intermediate situation may be envisaged in which two of the colour components are closely associated, but the third varies semi-independently. As a first step in the examination of this question, the data accumulated were used to compute the coefficients of correlation between the red, green, and blue intensities shown in Table I.

TABLE I
COEFFICIENTS OF CORRELATION BETWEEN COMPONENT INTENSITIES OF COLOUR OF
INDIVIDUAL SAMPLES

Quantities correlated	First sampling	Second sampling	Third sampling	All samplings
Initial intensity				
Red \times green	+ .43	+ .77	+ .81	+ .68
Red \times blue	+ .56	+ .42	+ .53	+ .51
Green \times blue	+ .61	+ .82	+ .87	+ .80
Stability				
Red \times green	+ .05	+ .20	+ .92	+ .85
Red \times blue	- .04	+ .27	+ .90	+ .80
Green \times blue	+ .57	+ .72	+ .94	+ .91
5% point	$\pm .30$	$\pm .30$	$\pm .30$	$\pm .16$

Considering first the coefficients for initial intensity at each time of sampling, given in the upper portion of Table I, all 12 of these are statistically significant, indicating that there is some real association between the component intensities of any particular sample. It is to be observed, however, that the degree of association between both red and green, and red and blue, is in no instance very high, and when the measurements for all samplings are considered collectively, is indeed quite moderate. Determination of red intensity alone consequently would not provide a highly accurate index of either the green or blue observed in the same sample, and vice versa. The association between green and blue is, however, appreciably stronger, whether the three samplings are considered individually or collectively.

In considering the observations of colour stability, or more correctly, instability, which is the property actually measured, it requires to be noted that the changes in colour on exposure after smoking (third sampling) were

much greater than those observed on either of the other two occasions, and consequently dominate the results when these are considered collectively. For this reason, in spite of the insignificant correlation between red and green and red and blue at the first two samplings, the results as a whole indicate an association between the changes in intensity which is moderately high in the case of red and green and red and blue, and quite high in the case of green and blue.

The question next arises as to whether the residual variance not accounted for by the foregoing correlations may be regarded as arising solely from experimental errors, or whether there are genuine independent fluctuations in the individual intensities from sample to sample. In the absence of any direct estimate of experimental error (single determinations only having been made on each sample) it is necessary to bring other evidence to bear on this point. This was done in two stages as follows:

The measurements of initial red, green, and blue intensity at all sampling times (396 observations) were first subjected to an analysis of variance (2) in which the main components of variation, arising from average differences between sides, between the intensity of the three colours, between the three sampling times, and the first-order interactions of these, were segregated from the second-order interactions of sides, sampling times, and individual colour intensities. As is pointed out by Fisher (3, sec. 41), such second-order interactions may be expected to be as a rule unimportant, and the variance apparently ascribable to them may accordingly be used to provide an estimate of experimental error, which will, however, be subject to inflation in proportion to the magnitude of any real interaction effects included in it.

When this was done, it was found that the residual variance of both green and blue, after correlation with red, was significantly greater than the foregoing interaction mean square, as judged by the usual variance ratio test (4). It would seem, therefore, that detectable variations in the intensity of blue and green, independent of red, may occur. The residual variance of blue after correlation with green did not exceed the interaction mean square, but owing to the possible inclusion of components other than error in the latter, this result is to be regarded as inconclusive, rather than definitely negative. In order to resolve this point, it is necessary to refer to computations, to be described in more detail in the next paper of this series, in which the homogeneity of the covariance of the green and blue intensity of individual samples within and between plants at each sampling time was examined in a manner described by Snedecor (4, sec. 12.3). By this means, significant variations in the blue intensity of samples from different plants, independent of concomitant variations in green, were demonstrable. It must be concluded, therefore, that there is in fact some element of independent variation in the intensity of each of the individual colours.

When examined in the same way, the measurements of change in colour on exposure yielded results paralleling the foregoing in that the residual variance of the change in both green and blue, after correlation with red, significantly exceeded the second-order interaction, and that the residual variance of change in blue after correlation with change in green did not. In this case, however, the subsequent analysis of covariance did not demonstrate significant differences in blue change between the product of different plants, independent of change in green.

In view of these facts, it would seem that in the investigation of an extended series of samples, measurements should be made in all three spectral regions in order to specify colour quality, particularly if it is desired to relate this to chemical or other factors. If only a limited number of samples is involved, the measurement of one component alone might suffice for comparative purposes at the present stage of development of the apparatus. For routine purposes such as plant control, it likewise seems probable that the determination of a single component might prove to be adequate, although a decision in this connection must depend on the correlation of the results of instrumental analyses with those of visual inspection. This point is currently receiving attention.

Colour Intensity or Brightness

As intimated above, the derivation of a satisfactory criterion of visual brightness from the instrumental readings made presents certain difficulties. As in the case of colour quality, it was not known beforehand whether the measurement of all three colour components was necessary, or whether one, or possibly two, of them would prove to be a satisfactory index of the total intensity. It is true that, in earlier papers, the relative brightness of different samples was assumed to be proportional to the intensity of red scatter alone, but the results then available were inadequate to demonstrate the small but significant element of independent fluctuation of each of the individual components described in the preceding section. On the other hand, in spite of this circumstance, certain considerations favour the use of the red intensity alone. One of these, touched upon already, is the probable tendency of visual inspection to assess the brightness from the intensity of the predominant red colour. Another is the fact that in addition to the independent differences noted above, there is also some degree of association between the intensities of the component colours of individual samples.

In the comparison of a limited number of samples, therefore, the red intensity may yield a satisfactory estimate of total brightness. If large numbers of samples are to be studied, as in the present investigation, the sum of the three components would seem to be preferable. A few measurements made with the apparatus without filters gave results about one-third the sum of the three percentage components obtained with filters, suggesting that the bright-

ness indicated by the latter is proportional to the intensity of reflection to be expected from a light source of the quality of a tungsten filament lamp. It is, of course, arguable that a still better index of brightness would result from a summation in which the three percentages were weighted in proportion to the absolute intensities of red, green, and blue characteristic of the incident light.

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CANADIAN WILTSHIRE BACON

VIII. COLOUR OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES¹

BY C. A. WINKLER², J. W. HOPKINS³, AND M. W. THISTLE⁴

Abstract

Photoelectric measurements on two factory-cured Wiltshire sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between individual sides in respect of both total intensity and quality of colour, which would seem to have arisen mainly from differences between plants. The average range of variation between plants was: total intensity, 25%; red intensity, 23%; green, 30%; and blue, 35% of the mean. Differences in colour quality of two types, (i) due to variations in the component intensities which were correlated but not of the same absolute magnitude, and (ii) due to uncorrelated variation in the component intensities, were demonstrable.

Partial correlation studies led to the deduction of a moderate degree of association between colour quality, and pH and nitrite content, under the conditions of sampling (ii). Increased acidity was accompanied by an enhanced green and a depressed blue intensity. Increased nitrite content also tended to depress blue intensity, but apparently without significantly affecting the green. No correlation between colour and the salt, nitrate or moisture content of the meat was demonstrable.

Introduction

In this paper, some of the colour measurements made during the course of a survey of factors influencing the quality of Canadian Wiltshire bacon (1) will be discussed. As intimated in the preceding paper of this series (7), determinations were made on samples from two sides from each of 22 Canadian packing plants, or 44 sides in all. Each side was sampled, by the procedure described elsewhere (1) on three occasions, namely, (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C. The resulting 132 samples were examined in a photoelectric colour comparator (6), by means of which separate measurements were made of the intensity of the light in the blue (4,000–4,500Å), green (4,900–5,800Å), and red (5,750–7,000Å) spectral regions reflected, or more correctly, scattered, at right angles to the surface of the meat. The results secured thus fall under the two heads of total intensity or brightness, and chroma or colour quality, of which the latter is considered to be the more important for reasons already put forward (7).

¹ Manuscript received February 23, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 41 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 912.

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In addition to the determination of colour at the actual time of sampling, measurements were also made of colour stability, as indicated by the change in colour of each sample after exposure for 20 hr. at 10° C. and 95% relative humidity. These will form the subject of a separate communication.

Colour Intensity or Brightness

It has already been shown (7) that there is some element of independent variation in the amounts of red, green, and blue scatter from sample to sample, and that measurements of all three should consequently be used in order to specify both total intensity and quality of colour. The figures for total intensity dealt with in this section are accordingly the sums of these three components in each case, although even these, for reasons indicated elsewhere (7), are probably not entirely satisfactory indices of visual brightness.

Analysis of Variance

The variance of the 132 observations of intensity or total brightness, as defined above, was analysed, by the procedure due to Fisher (5), into three portions, ascribable (i) to average differences, over all plants, between the three sampling occasions, (ii) to average differences, over the three samplings, between sides, and (iii) to the differential variation, or interaction, of sides with sampling time. The variance between sides was further allocated within and between plants, following which a corresponding partition of the interaction variance was also made. The results of this computation are shown in Table I, while Table II summarizes some of the main features of the actual observations.

TABLE I
ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR BRIGHTNESS

Variance due to	Degrees of freedom	Mean square
Sides	43	76.3*
Between plants	21	97.2
Within plants	22	56.3
Sampling times	2	647.1**
Interaction sides × samplings	86	47.1
Between plants	42	55.0
Within plants (residual)	44	39.6

* Exceeds mean square residual, 5% level of significance.

** Exceeds mean square residual, 1% level of significance.

The analysis of variance indicates a definitely significant difference between sampling times in the average measurements for all 44 sides, due, however, almost entirely to the increased reflectivity of the samples taken after smoking (sampling (iii)). Moderately significant differences in the average bright-

TABLE II

SUMMARY OF MEASUREMENTS OF COLOUR INTENSITY OR BRIGHTNESS

Quantity	Sampling time		
	(i)	(ii)	(iii)
Maximum			
Individual side	104.5	102.5	110.7
Plant (av. 2 sides)	96.2	97.4	108.6
Minimum			
Individual side	74.8	74.2	72.9
Plant (av. 2 sides)	79.4	75.4	78.2
Mean	87.8	88.1	94.6

ness over all three samplings of individual sides are also demonstrable. These would seem to have arisen mainly from differences between plants, as although the number of observations available is not sufficient to establish the statistical reality of the difference in the inter- and intra-plant variance, the variance between sides drawn from the same plant does not significantly exceed the residual. This is a point of some interest, since the variability of sides from the same plant probably arises mainly from inherent differences in carcasses prior to curing, whereas between plants, differences in curing practice may be operative. On the other hand, there seems to have been no pronounced differential effect of smoking on the total colour intensity of the product from different plants, the interaction of plants \times samplings (Table I), although suggestive, failing significantly to exceed the residual variance.

The means for the three samplings, shown in Table II, all fall approximately midway between the respective maximum and minimum plant averages, indicating that the values for the 22 individual plants were reasonably symmetrically distributed over the observed range of variation, without any marked bias in either direction. The average range between the maximum and minimum plant averages in the three samplings was 25% of the mean. Although doubtless susceptible of some improvement, this may perhaps be regarded as not unreasonable in view of the fact that the averages were, after all, deduced from only two sides from each plant, and further that the product of different plants was of varying age when received at the laboratory.

Correlation with Chemical Analyses

In order to ascertain whether there was any readily demonstrable association between total intensity of colour and chemical composition as determined by Cook *et al.* (2, 3), a number of simple correlation coefficients were calculated. With one exception, these proved to be uniformly insignificant. The results for sampling (iii), i.e., after smoking, yielded a coefficient of $r = -0.38$ between colour intensity and nitrite concentration in p.p.m. (5% point, $r = \pm 0.30$), but the coefficients for both the earlier samplings were insignificant.

nificant. Nitrate and moisture content also gave insignificant coefficients for all three samplings. Salt concentration and pH were examined only for the first two samplings. Neither was correlated with intensity. The partial correlation between intensity and pH, after allowing for associated variations in nitrite concentration, was likewise insignificant for both samplings (i) and (ii).

Chroma or Colour Quality

Analysis of Variance and Covariance of Component Intensities

Table III gives the results of an analysis of variance of the component red, green, and blue intensities of the individual samples paralleling that for total brightness. The previously noted difference in total intensity between sampling times is seen to be the result of variation in all three components. Table IV shows, however, that the increase after smoking was greatest in the blue region of the spectrum and least in the red. The analysis of variance likewise indicates that the observed differences between sides in respect of red scatter were no greater than would be expected from the residual variance in this respect of successive samples from the same side, which latter, however, is sensibly greater than the corresponding residual variance of either green or blue scatter. On the other hand, the differences between sides in both green and blue are quite significant, and the inter-plant variance of blue demonstrably exceeds the intra-plant. It is also to be noted that the increase in intensity of green and blue after smoking was not of the same magnitude in the product from all plants, the interaction of plants \times samplings (Table III) being significant in both instances. A complicating factor must be noted here, however, in that the sides from the eastern and western plants were smoked on different occasions, and apparently under somewhat different conditions.

TABLE III
ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	10.6	8.3**	13.8**
Between plants	21	11.3	10.6	24.0
Within plants	22	9.7	6.0	4.1
	2	34.9*	68.7**	122.4**
Sampling times	86	10.2	5.3	5.1**
Interaction sides \times samplings				
Between plants	42	9.7	6.9*	8.3**
Within plants (residual)	44	10.6	3.8	1.9

* Exceeds mean square residual, 5% level of significance.

** Exceeds mean square residual, 1% level of significance.

TABLE IV

SUMMARY OF MEASUREMENTS OF COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum									
Individual side	48.6	32.7	29.2	46.6	30.1	30.0	44.4	34.0	33.3
Plant (av. of 2 sides)	41.2	31.2	26.2	43.8	29.0	29.0	43.2	32.9	32.6
Minimum									
Individual side	30.4	22.1	20.0	30.2	22.0	19.9	32.9	21.9	20.0
Plant (av. of 2 sides)	33.0	23.4	20.6	33.2	22.2	19.9	34.0	23.3	20.9
Mean	38.0	26.6	23.2	37.8	26.1	24.1	39.4	28.5	26.4

The means of each component intensity again fall about mid-way between the respective maximum and minimum plant averages in all three samplings. The range between plants, averaged over the three samplings, is 23% in red, 30% in green, and 35% in blue.

Reference has already been made (7) to the covariance of the component intensities from sample to sample of meat. In view of this, two types of variation in colour quality are possible: (i) within a homogeneous system, due to the fact that the increments in the component intensities associated with a given increase in total brightness, although correlated, are not of the same magnitude; and (ii) of an irregular nature, due to uncorrelated variations in the component intensities.

It was shown (7) that variations in colour quality of type (ii) were demonstrable, and in this connection an analysis of covariance was employed to establish the fact that there were significant differences in the blue intensity of the product from different plants, independent of associated variations in green, on all three sampling occasions. A further analysis of the covariance of the red and blue intensities of the six samples (from two sides at each of three sampling times) of product from each plant into portions within and between samplings (3 and 2 d.f. for each of 22 plants respectively) was also made. This indicated that there were significant differences in blue intensity, independent of red, between samplings.

These analyses also provided information respecting the effect on colour quality of the correlated, as well as the uncorrelated, variations in component intensities. Thus, the regression coefficients within plants calculated in the course of the first analysis indicated an average change of 0.67 units in blue intensity associated with unit change in green, and those within samplings determined in the second analysis, an average change of 0.31 units of blue per unit of red. It must be concluded, therefore, that variations in colour quality of both the types mentioned above occur in practice, and that those of type (ii) may be encountered either in the product of different plants, or in that of the same plant sampled before and after smoking.

Correlation of Component Intensities with Chemical Analyses

As a first step in the investigation of the relation between the component intensities of colour and the chemical properties of the meat, the simple correlation coefficients listed in Table V were determined. These indicate that above-average nitrite content was associated with below-average intensities of both green and blue. Otherwise, they are quite insignificant, with the exception of those for blue intensity and pH (sampling (ii)), and blue intensity and moisture content (all data).

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour measurement	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
sampling (i)	-.03	-.18	-.00	-.06	-.06
sampling (ii)	-.06	-.13	+.06	+.01	-.03
sampling (iii)	—	+.08	-.21	—	+.18
All data	+.05	-.05	-.05	-.02	-.12
Green intensity					
sampling (i)	-.14	-.22	-.32*	-.41	-.12
sampling (ii)	-.13	-.19	-.09	+.17	+.02
sampling (iii)	—	-.06	-.38*	—	+.16
All data	-.18	-.11	-.28**	-.09	-.20
Blue intensity					
sampling (i)	+.01	-.02	-.14	-.26	-.25
sampling (ii)	-.15	-.21	-.20	+.40**	-.02
sampling (iii)	—	-.13	-.41**	—	+.21
All data	+.05	-.07	-.29**	+.05	-.31**

* Exceeds 5% level of significance.

** Exceeds 1% level of significance.

It has to be recognized, however, that simple correlation coefficients may fail to portray adequately the relation between colour quality and chemical composition. In the first place, the former is dependent in part on the general level of intensity, owing to the differential magnitude of the correlated variations in the three spectral components. Secondly, as has been pointed out by Cook and White (4), fluctuations from sample to sample in the chemical factors themselves are not all mutually independent. Both of these circumstances may operate either to obscure or to exaggerate the real relation between individual colour components and chemical qualities. For this reason, a number of third order partial correlation coefficients have been calculated, in order to examine the relation between pairs of factors independent of associated variation in certain others. These, which are confined to the results for samplings (i) and (ii), i.e., before smoking, will be found in Table VI.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated		Independent of		Correlation coefficient	
				Sampling (i)	Sampling (ii)
<i>Intensity</i>	<i>Analysis</i>	<i>Analysis</i>	<i>Intensity</i>		
Red	× salt	Nitrate,	blue and green	+ .03	+ .03
		Nitrite,	blue and green	— .09	+ .00
		Moisture,	blue and green	+ .04	— .04
		pH,	blue and green	—	+ .05
Red	× nitrate	Salt,	blue and green	— .18	— .01
		Nitrite,	blue and green	—	+ .01
		Moisture,	blue and green	— .16	—
Red	× nitrite	Salt,	blue and green	+ .17	+ .10
		Nitrate,	blue and green	—	+ .10
		pH,	blue and green	+ .07	+ .06
Red	× pH	Salt,	blue and green	—	+ .12
		Nitrite,	blue and green	+ .10	+ .08
Red	× moisture	Salt,	blue and green	+ .10	— .14
		Nitrate,	blue and green	— .00	—
Green	× salt	Nitrate,	red and blue	— .12	— .04
		Nitrite,	red and blue	— .04	— .03
		Moisture,	red and blue	— .21	— .02
		pH,	red and blue	—	— .09
Green	× nitrate	Salt,	red and blue	— .19	— .02
		Nitrite,	red and blue	—	— .02
		Moisture,	red and blue	— .26	—
Green	× nitrite	Salt,	red and blue	— .27	— .00
		Nitrate,	red and blue	—	— .01
		pH,	red and blue	— .17	+ .18
Green	× pH	Salt,	red and blue	—	— .28
		Nitrite,	red and blue	— .21	— .30
Green	× moisture	Salt,	red and blue	— .10	+ .15
		Nitrate,	red and blue	— .11	—
Blue	× salt	Nitrate,	red and green	+ .07	— .07
		Nitrite,	red and green	+ .13	— .01
		Moisture,	red and green	— .02	— .12
		pH,	red and green	—	+ .05
Blue	× nitrate	Salt,	red and green	+ .18	— .10
		Nitrite,	red and green	—	— .11
		Moisture,	red and green	+ .10	—
Blue	× nitrite	Salt,	red and green	— .04	— .12
		Nitrate,	red and green	—	— .15
		pH,	red and green	+ .07	— .35*
Blue	× pH	Salt,	red and green	—	+ .45**
		Nitrite,	red and green	— .11	+ .53**
Blue	× moisture	Salt,	red and green	— .21	— .17
		Nitrate,	red and green	— .15	—

* Exceeds 5% point ($r = 0.31$).** Exceeds 1% point ($r = 0.40$).

They are again, for the most part, of quite negligible magnitude. Demonstrable effects are confined to the blue region of the spectrum, in which the intensity seems to be correlated negatively with nitrite content and positively with pH, i.e., the blue component increased with increasing alkalinity. There is also some indication of a negative association between green intensity and pH, but this falls short of significance. It is noteworthy that the main colour component, red, yields no indication of association with any of the chemical quantities determined.

In view of the indications obtained, the correlation of blue and green intensities with nitrite content and pH was further investigated by the calculation of still higher order partial coefficients from the results of sampling (ii). When this was done, the fourth order coefficient for green intensity and pH, independent of red intensity, blue intensity, salt content, and nitrite content, was found to be $r = -0.33$, which just exceeds the 5% point of $r = \pm 0.31$. Similarly, the fourth order partial correlation of blue intensity and pH, independent of red intensity, green intensity, salt content, and nitrite content, gave $r = 0.56$, and the fifth order partial correlation between blue intensity and nitrite content, independent of red intensity, green intensity, salt content, nitrate content, and pH, $r = -0.43$, both of which exceed the 1% point. There would thus seem to have been, under the conditions of sampling (ii) at any rate, a moderate degree of association between these two factors and colour quality, such that increasing acidity tended to raise the green and depress the blue intensity, and increasing nitrite content also tended to depress the blue intensity, but without affecting the green.

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CANADIAN WILTSHIRE BACON

IX. COLOUR STABILITY OF BACON AND ITS CORRELATION WITH CHEMICAL ANALYSES¹

BY C. A. WINKLER², J. W. HOPKINS³, AND E. A. ROOKE⁴

Abstract

Photoelectric measurements on samples of two factory-cured sides from each of 22 Canadian packing plants, sampled (i) upon receipt at the laboratory, (ii) after storage for 10 days at 1° C., and (iii) after smoking for 14 hr. at 40° C., indicated statistically significant differences between the product of individual plants in respect of the stability of total intensity of colour, and of the component intensities of red, green, and blue, on exposure of freshly-cut samples for 20 hr. at 10° C., and 95% relative humidity. Apart from a batch effect after smoking, however, there was no marked segregation of any one plant or group of plants from the remainder in respect of colour stability of product. On the average, the effect of smoking was to reduce colour stability.

Analyses of covariance demonstrated (i) a significant degree of correlation between the green and blue stability of the same sample, and (ii) a further correlation between the initial green and blue intensity and the stability of these components, samples of higher initial intensity suffering a greater reduction on exposure. Partial correlation studies suggest that increased nitrite content was accompanied by an enhanced stability of the red component of colour, but no correlation between the salt, nitrate and moisture content or pH of the meat and its colour stability was demonstrable.

In the preceding paper of this series (7), some of the colour measurements made in a survey of factors influencing the quality of Canadian Wiltshire bacon (1) were discussed. It was explained that observations were made on samples from 44 sides, two from each of 22 Canadian packing plants, each side being sampled (i) upon receipt at the laboratory; (ii) after storage for 10 days at 1° C.; and (iii) after smoking for 14 hr. at 40° C. The measurements of colour stability, now to be discussed, were secured by determining the change in intensity and quality of colour of the 132 individual samples after exposure for 20 hr. at 10° C. and 95% relative humidity. Apparatus and procedure were the same as those already described (6, 7), and the results will be dealt with under the heads previously adopted in considering the measurements of initial colour.

Change in Colour Intensity or Brightness

For reasons advanced elsewhere (6), total intensity or brightness is defined as the sum of the separate intensities of the red, green, and blue spectral components, relative to the white standard.

¹ Manuscript received February 23, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 42 of the Canadian Committee on Storage and Transport of Food, and as N. R. C. No. 913.

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Analysis of Variance

The 132 individual measurements of change in total intensity or brightness as defined above, were subjected to an analysis of variance (5), of the same form as that applied to the observations of initial colour. The results of this are given in Table I, while Table II summarizes salient features of the measurements themselves. It will be observed that the mean values in Table II are all negative in sign, indicating an average decrease in intensity of colour of the samples on exposure.

TABLE I
ANALYSIS OF VARIANCE OF CHANGE IN TOTAL INTENSITY OF COLOUR

Variance due to	Degrees of freedom	Mean square
Sides	43	39.8**
Between plants	21	71.7**
Within plants	22	9.4
Samplings	2	3064.5**
Interaction sides \times samplings	86	68.9**
Between plants	42	128.3**
Within plants (residual)	44	12.2

** Exceeds mean square residual, 1% level of significance.

From the analysis of variance, it is to be deduced that there were significant differences in the colour stability of the product of different plants, but that the variance between sides from the same plant did not exceed the residual. On the average, stability changed significantly between samplings, but the interaction mean square indicates that the product of individual plants behaved differentially in this respect. In reality, the variance between samplings is very largely due to the decrease in average colour stability after smoking

TABLE II
SUMMARY OF MEASUREMENTS OF CHANGE IN TOTAL INTENSITY OF COLOUR

Quantity	Sampling		
	(i)	(ii)	(iii)
Maximum change			
Individual side	-12.2	-11.3	-35.7
Plant (av. 2 sides)	-10.2	- 8.0	-34.4
Minimum change			
Individual side	+ 0.1	+ 0.1	- 0.8
Plant (av. 2 sides)	- 0.1	+ 0.1	- 1.4
Mean change	- 4.7	- 1.7	-17.4

(Table II), and the differential effect is likewise largely ascribable to the fact that this decrease was confined to the sides from the eastern plants, in which it was very pronounced, and absent from those from the western plants, which were smoked in two separate batches. Unfortunately, it cannot be stated whether this discrepant behaviour was due to differences in age or other inherent properties of the meat at the time of smoking, or to variation in the conditions under which the two batches were smoked. Examination of the individual observations in samplings (i) and (ii), however, does not suggest that any one plant or group of plants stood out from the remainder in respect of colour stability of product before smoking. Rather, there was a continuous series of plant averages from the maximum to the minimum observed values.

Correlation of Change with Initial Intensity

The correlation of the observed change in total intensity of colour on exposure with the initial intensity of the freshly cut samples was also investigated, for each sampling separately. The 44 individual samples in sampling (i) gave $r = -0.28$, and those in sampling (ii) $r = -0.18$, both of which fall short of the 5% point ($r = \pm 0.30$). On the other hand, the results for sampling (iii) as a whole gave $r = +0.40$, exceeding the 1% point of $r = \pm 0.38$. This, however, was again due to the fact that the sides from the eastern plants, smoked in the first batch, exhibited both a lower average initial intensity of colour, and also an appreciably greater average decrease in intensity on exposure. So much was this the case, that when the covariance was computed about the two batch means separately, rather than about the mean of all 44 samples, a significantly negative value of $r = -0.33$ was obtained, indicating that within batches, those samples having the highest initial intensity actually tended, on the average, to show the greatest decrease on exposure.

Correlation with Chemical Analyses

Simple correlation coefficients were calculated between the change in total intensity and the salt content, nitrate content, nitrite content, moisture content, and pH of individual sides, as determined by Cook, White, and Chadderton (2, 3), but in view of the differential behaviour of the eastern and western batches after smoking, these computations were confined to the results for the first two samplings.

The resulting coefficients were all insignificant with the exception of the one for nitrite content in sampling (ii). This gave $r = +0.37$, which exceeds the 5% point of $r = 0.30$ and thus suggests that increased nitrite content may be associated with stability of colour. The corresponding value for sampling (i), $r = +0.23$, failed to attain significance, but that for sampling (ii), at $+0.39$, was practically unaffected when recomputed as a first-order partial, eliminating the effect of accompanying variations in pH.

Changes in Chroma or Colour Quality

Analysis of Variance and Covariance of Change in Component Intensities

Tables III and IV show respectively the analysis of variance and range of fluctuation of the changes on exposure, in the individual intensities of red, green, and blue. All reproduce the main features already noted in the observations of total intensity, although the differences between plants in respect of blue stability are less pronounced than those in the red and green regions. Here also the interpretation of the differential behaviour of the sides from the eastern and western plants after smoking is obscured by batch effects.

The covariance of the changes in red, green, and blue intensity in individual samples has already been discussed to some extent in an earlier paper, which contained a tabulation of the correlation coefficients bearing upon this point (6, Table I). The results for samplings (i) and (ii) agreed in indicating

TABLE III
ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Variance due to	D.f.	Mean square		
		Red	Green	Blue
Sides	43	7.6**	7.0**	3.5
Between plants	21	11.2**	12.9**	5.3*
Within plants	22	4.1	1.4	1.8
Samplings	2	505.2**	390.2**	175.4**
Interaction sides × samplings	86	13.2**	9.8**	5.3*
Between plants	42	23.6**	17.5**	7.9**
Within plants (residual)	44	3.3	2.5	2.9

* Exceeds mean square residual, 5% level of significance.

** Exceeds mean square residual, 1% level of significance.

TABLE IV
SUMMARY OF MEASUREMENTS OF CHANGE IN COMPONENT INTENSITIES OF COLOUR

Quantity	Sampling (i)			Sampling (ii)			Sampling (iii)		
	Red	Green	Blue	Red	Green	Blue	Red	Green	Blue
Maximum decrease									
Individual side	-10.7	-3.8	-7.0	-6.7	-2.9	-3.5	-17.3	-11.7	-8.5
Plant (av. 2 sides)	-7.6	-2.4	-4.0	-5.4	-1.4	-2.8	-15.9	-10.8	-8.2
Minimum decrease									
Individual side	-0.4	+4.1	+2.6	+1.7	+2.5	+2.3	-1.0	+1.7	+0.5
Plant (av. 2 sides)	-0.8	+3.1	+1.2	+0.1	+2.0	+1.7	-1.4	+0.9	+0.1
Mean change	-3.8	-0.1	-0.8	-2.1	+0.5	-0.1	-8.6	-5.0	-3.8

a significant positive correlation between the changes in green and blue, but did not demonstrate any definite association of either of these quantities with red stability. Changes in all three intensities were significantly correlated in sampling (iii), but this must again be considered as a reflection of the batch effect mentioned above. It was also pointed out that there were significant variations in the stability of blue and green, independent of that of red, in the observations as a whole. This was, however, found to be no more pronounced in the sides from different plants than in those from the same plant, with the exception of green stability in sampling (i), which showed some variation from plant to plant independent of red.

A further analysis of the covariance of the red and green, and red and blue stability of the six samples (two sides at each of three sampling times) taken from the product of each plant, into portions within and between samplings, was also made. This paralleled a similar analysis of the actual intensities at the time of sampling, reported in the preceding paper (5). The results demonstrated (i) no significant element of correlation between the stability of the three colour components of individual samples of the bacon from the same plant, within samplings, but (ii) significant differences between samplings in the stability of both blue and green, independent of red stability. This effect was, however, confined to the samples from the eastern plants, and must accordingly be regarded as a further consequence of batch differences.

Correlation with Components of Initial Intensity

As in the case of total intensity, the correlation between the stability of red, green, and blue, and the initial intensity of these components of colour in the fresh samples was determined for each sampling.

The observations of initial red and change in red intensity in the two samplings prior to smoking gave $r = -0.21$ for sampling (i) and $r = +0.05$ for sampling (ii), both of which are statistically insignificant. For green, the values of r for the two samplings were -0.63 and -0.30 . As the first of these exceeds the 1%, and the second attains the 5% point, both may be regarded as significant, and indicative of the fact that samples above average in initial green intensity tended to suffer a correspondingly greater reduction in this component of colour on exposure. A similar conclusion is to be drawn from the coefficients of -0.51 and -0.48 obtained from the measurements of blue intensity.

For sampling (iii), the observations in the red, green, and blue regions gave $r = -0.03$, $+0.36$, and $+0.50$ respectively over all samples, but these results were again clearly biased by batch differences, recomputations of the covariance about the batch means yielding $r = -0.31$, -0.42 , and -0.39 . Within batches therefore the indications are that, on the whole, greater decreases in each of the three component intensities occurred in those samples having the higher initial values.

Correlation with Chemical Analyses

In view of the anomalous circumstances attending sampling (iii), the correlation of the colour stability of individual sides with the results of chemical analyses was confined to samplings (i) and (ii). Even in these instances, it is necessary to take cognizance of possible complicating effects due to the association between the initial intensity of blue and green and the subsequent stability of these components, but it will be convenient first to examine the observed correlation between colour stability and the various chemical constituents studied, and then to determine whether any observed effects are to be regarded as due to the operation of the chemical factors directly upon stability, or indirectly through their influence on initial colour.

TABLE V

COEFFICIENTS OF SIMPLE CORRELATION BETWEEN CHANGES IN COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Colour change	Salt content	Nitrate content	Nitrite content	pH	Moisture content
Red intensity					
Sampling (i)	+ .06	+ .05	− .07	− .16	− .19
Sampling (ii)	+ .17	− .11	+ .38*	− .09	− .06
Both samplings	+ .28*	+ .03	+ .17	− .21*	− .32**
Green intensity					
Sampling (i)	+ .19	+ .10	+ .36*	+ .32*	+ .10
Sampling (ii)	+ .10	+ .09	+ .26	+ .08	+ .01
Both samplings	+ .22*	+ .11	+ .34**	+ .16	− .05
Blue intensity					
Sampling (i)	− .04	− .02	+ .22	+ .25	+ .20
Sampling (ii)	− .02	+ .06	+ .25	+ .04	+ .32*
Both samplings	+ .09	+ .02	+ .26*	+ .09	+ .05

* Exceeds 5% level of significance.

** Exceeds 1% level of significance.

Of the various simple correlation coefficients shown in Table V, those of nitrite content with green and blue stability alone exhibit any measure of persistency both within and between samplings. The apparent correlation in the data as a whole between red stability on the one hand, and salt content, pH, and moisture content on the other, is not in evidence within samplings, and hence may be due to differences in the means for the two samplings, rather than to an actual causal association. There is, however, some indication of an effect of nitrite on red stability in sampling (ii). In this, as in the other instances in which a significant effect of nitrite was demonstrable, an above average concentration of this substance was associated with increased colour stability.

TABLE VI

COEFFICIENTS OF PARTIAL CORRELATION BETWEEN STABILITY OF COMPONENT COLOUR INTENSITIES AND CHEMICAL ANALYSES OF BACON

Quantities correlated		Independent of		Correlation coefficient	
				Sampling (i)	Sampling (ii)
<i>Change</i>	<i>Analysis</i>	<i>Analysis</i>	<i>Change</i>		
Red	× salt	Nitrate, blue and green		+.02	+.15
		Nitrite, blue and green		+.08	+.09
		Moisture, blue and green		-.05	+.12
		pH, blue and green		—	+.16
Red	× nitrate	Salt, blue and green		+.03	-.09
		Nitrite, blue and green		—	-.05
		Moisture, blue and green		-.03	—
Red	× nitrite	Salt, blue and green		-.12	+.30
		Nitrate, blue and green		—	+.32*
		pH, blue and green		-.00	+.39*
Red	× pH	Salt, blue and green		—	-.07
		Nitrite, blue and green		-.16	-.23
Red	× moisture	Salt, blue and green		-.14	-.09
		Nitrate, blue and green		-.13	—
Green	× salt	Nitrate, blue and red		+.23	+.18
		Nitrite, blue and red		+.13	+.14
		Moisture, blue and red		+.30	+.01
		pH, blue and red		—	+.19
Green	× nitrate	Salt, blue and red		+.06	+.11
		Nitrite, blue and red		—	+.09
		Moisture, blue and red		+.15	—
Green	× nitrite	Salt, blue and red		+.22	+.08
		Nitrate, blue and red		—	+.14
		pH, blue and red		+.22	+.10
Green	× pH	Salt, blue and red		—	+.11
		Nitrite, blue and red		+.09	+.03
Green	× moisture	Salt, blue and red		+.17	-.30
		Nitrate, blue and red		+.07	—
Blue	× salt	Nitrate, red and green		-.16	-.17
		Nitrite, red and green		-.20	-.18
		Moisture, red and green		-.10	+.06
		pH, red and green		—	-.17
Blue	× nitrate	Salt, red and green		-.04	-.02
		Nitrite, red and green		—	+.04
		Moisture, red and green		-.01	—
Blue	× nitrite	Salt, red and green		+.09	+.08
		Nitrate, red and green		—	+.04
		pH, red and green		-.04	+.04
Blue	× pH	Salt, red and green		—	-.04
		Nitrite, red and green		+.09	-.01
Blue	× moisture	Salt, red and green		+.07	+.45**
		Nitrate, red and green		+.14	—

* Exceeds 5% point ($r = 0.31$).** Exceeds 1% point ($r = 0.40$).

As was pointed out in the previously published discussion of initial colour (5), however, simple correlation coefficients may fail to provide an adequate representation of the actual underlying relations, owing to the mutual inter-correlation, in individual samples, of (i) certain of the chemical constituents (2), and (ii) of the green and blue stability, as well as (iii) of stability and initial intensity. The analysis of these relations was accordingly pursued further by the calculation of the third order partial correlation coefficients listed in Table VI. These provide a measure of the correlation between the concentration of each chemical and the stability of each component colour intensity, after making allowance for the interrelation of both quantities under consideration with the two other component intensities of colour, as well as with one additional chemical factor.

The results now suggest that the direct effect of nitrite content on colour stability was confined to the red region of the spectrum, and that the apparent correlation of this factor with green and blue stability, noted above (Table V), was due to the interrelations mentioned. The only other significant coefficient in Table VI is that between blue stability and moisture content, independent of red stability, green stability, and salt content, in sampling (ii). Salt content itself seems to have been without measurable effect on either colour or colour stability, and pH, which appeared to have a significant influence upon initial blue intensity, likewise seems to have been without effect on the subsequent stability of this component.

The foregoing inference respecting the influence of nitrite content on red change was strengthened by the calculation of the sixth order partial correlation coefficient between these quantities, independent of salt content, nitrate content, pH, initial red intensity, green change, and blue change, which was found to be 0.43. This is the more noteworthy, since the demonstrable effects of nitrite on initial colour were confined to the blue region (5). On the other hand, the apparent association between blue stability and moisture content noted above would seem to have been spurious, as the fourth order coefficient, independent of initial blue intensity, was reduced to the insignificant level of $r = -0.09$. The absence of correlation between green change and the chemical constituents studied was confirmed by the calculation of fourth order coefficients, independent of initial green intensity, which proved to be uniformly insignificant.

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THE NITROGENOUS CONSTITUENTS OF CAT'S SUBMAXILLARY SALIVA EVOKED BY PARASYMPATHETIC AND SYMPATHETIC STIMULATION¹

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Abstract

The nitrogenous constituents of submaxillary saliva were studied in samples of saliva secreted by cats in response (i) to chorda tympani stimulation and (ii) to administration of adrenaline. Each of these two kinds of saliva was found to contain a different and characteristic glucoprotein. These two proteins are believed to be secreted by different cells, viz., by mucous cells in response to chorda tympani stimulation, and by serous cells in response to adrenaline. A new method is described for the determination of non-protein nitrogen in submaxillary saliva. Study of the partition of non-protein nitrogen showed that urea represents quantitatively the main fraction of the non-protein nitrogen of submaxillary saliva secreted in response to either parasympathetic or sympathetic stimulation. Prolonged chorda tympani stimulation causes a decrease in the permeability of the submaxillary gland to the passage of non-protein nitrogen, the fraction least affected being the urea nitrogen. The administration of adrenaline greatly increases the permeability to all fractions of non-protein nitrogen, especially urea, and this effect persists for several hours after the adrenaline administration is discontinued.

Introduction

Data concerning the protein material and non-protein nitrogenous substances in the saliva secreted by the submaxillary glands under various conditions of stimulation are very incomplete and fragmentary. As Langley (9) states in his review of the literature on salivary secretion, the chief organic material contained in the saliva from mucous (mixed) glands is mucin. Langley considered it possible that the mucin might be present in two forms, because whereas most of the mucin is precipitated by acetic acid in a stringy lump, part of it not infrequently takes the form of fine particles. He believed that some globulin is also present in the saliva of the mixed glands. The saliva from the serous glands contains globulin, or a body allied to globulin, alkali albuminate, and a small amount of serum albumin. Almost 30 years later, in a review of the same subject, Rosemann (18) was able to add very little to the above data. To quote his own words: "It seems that the protein of the saliva belongs to the class of albumins and globulins, but exact data are lacking. Similarly we know nothing about the chemical composition of the salivary mucus." Recently Kesztyüs and Martin (8) reported that the submaxillary saliva obtained on stimulation of the chorda tympani or the sympathetic nerve in the dog contains both mucin and albumin, and that the sympathetic saliva is richer in these substances than the chorda tympani saliva.

¹ Manuscript received February 7, 1940.

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As to the nature of the non-protein nitrogenous constituents of the saliva, the available data relate chiefly to human saliva, and largely to the mixed saliva of the mouth cavity. The following substances have been found to be normally present in the saliva: urea, ammonia, uric acid, creatinine, amino-nitrogen. Under pathological conditions, where there is retention of non-protein nitrogenous substances in the blood, the concentration of urea, ammonia, and uric acid in the saliva is increased. A review of the rather voluminous literature on the non-protein nitrogenous constituents of saliva is given by Babkin (1, 4).

The scarcity of data concerning both the nature and the concentration of protein and non-protein nitrogenous substances in the saliva obtained by stimulation of the parasympathetic or sympathetic nerves supplying the salivary glands moved us to re-investigate this problem. Recently we have been able to obtain sufficient quantities of saliva either by parasympathetic or sympathetic stimulation of the submaxillary glands in the cat to provide some data concerning both the nature of the protein material and the partition of non-protein nitrogen. We believe that the results reported here furnish some new data concerning the composition of different types of submaxillary saliva, and moreover that they warrant important conclusions as to the part played by the parasympathetic and sympathetic nerves in the activity of the submaxillary glands.

Methods

PHYSIOLOGICAL TECHNIQUE

Cats, anaesthetized with nembutal, were used in the experiments. The chorda tympani and cervical sympathetic nerves were cut on both sides and the submaxillary ducts cannulated. Rhythmic stimulation was applied to the nerves for 10-minute periods interrupted by 5-minute intervals of rest. Adrenaline was administered intravenously in some experiments as a substitute for sympathetic nerve stimulation. The physiological technique was very similar to that described in the papers of Hebb and Stavraky (7) and Langstroth, McRae, and Stavraky (10, 11). For the sake of comparison, some analyses were also made of parotid saliva, which was obtained from dogs with a permanent fistula of the parotid gland.

CHEMICAL ANALYSIS

For the quantitative estimation of non-protein nitrogen, the proteins of the saliva were precipitated as follows: Two volumes of acetone, containing 3% acetic acid, were thoroughly mixed with one volume of saliva. The precipitate was separated by centrifuging and washed twice with a solution consisting of two parts of acetone and one part of water, and saturated with sodium chloride. This was followed by washing with 95% alcohol, absolute acetone, a mixture of three volumes of absolute alcohol and one volume of ether, and finally with ether. The original supernatant fluid and the washings were combined and evaporated to dryness under reduced pressure

at 20°C. or below. The residue was dissolved in water so that 2 cc. of the solution was equivalent to 1 cc. of saliva. In this filtrate, urea and the nitrogenous volatile bases were determined by the urease-aeration-titration method of Van Slyke and Cullen (13, pp. 547-551) and creatine + creatinine by Folin's open flask method (13, pp. 582-585). Determination of nitrogen in the whole saliva and in the protein-free filtrates was carried out by the micro-Kjeldahl procedure of Pregl (14).

Results

The Protein Constituents of the Submaxillary Saliva

In order to study the precipitability of the protein constituents of the saliva we have tried various known protein precipitants. Of special interest are the results obtained with trichloroacetic acid and with acetone, which are presented in Table I. Acetic or hydrochloric acid in certain concentrations caused some precipitation in the submaxillary saliva obtained on stimulation of the chorda tympani, but the precipitation was never complete. The results obtained by precipitation of the saliva with two volumes of acetone containing 3% of acetic acid were of special interest. Not only was the precipitation of protein material always found to be complete, but the physical characteristics of the precipitates obtained from various kinds of submaxillary saliva, for example, (i) that secreted in response to chorda tympani stimulation (or to injection of parasympathomimetic drugs like pilocarpine), and (ii) that secreted in response to stimulation of the cervical sympathetic nerve (or to injection of adrenaline), were strikingly different. The appearance of these precipitates is illustrated in Fig. 1. The acetone precipitates obtained from sympathetic or adrenaline saliva were readily soluble in distilled water, dilute solutions of sodium carbonate, sodium and potassium hydroxide, and also in dilute solutions of hydrochloride acid, and formed non-

TABLE I

PRECIPITABILITY OF PROTEIN MATERIAL IN VARIOUS KINDS OF SALIVARY SECRETION BY TRICHLOROACETIC ACID AND ACETONE IN THE PRESENCE OF ACETIC ACID

Saliva	Trichloroacetic acid (5%, 7%, or 10%)	2 vol. acetone containing 3% acetic acid	Viscosity of alk., neutral, or acid soln. of acetone, ppt.
Parotid (dog)	Complete pptn.	Flocculent ppt.	Non-viscous
Submaxillary (cat), chorda tympani	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), pilocarpine	No ppt.	Ppt. in form of compact viscous masses, also slight turbidity.	Viscous
Submaxillary (cat), sympathetic	No ppt.	Flocculent ppt.	Non-viscous
Submaxillary (cat), adrenaline	No ppt.	Flocculent ppt.	Non-viscous

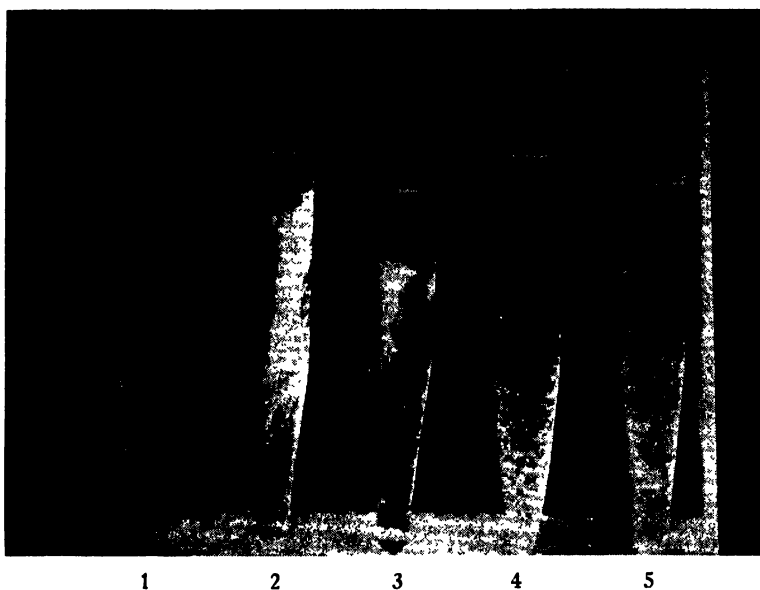


FIG. 1. *Appearance of the precipitates formed when various samples of cat's submaxillary saliva were poured into centrifuge tubes containing double the volume of 3% solution of acetic acid in acetone and the contents of each tube were thoroughly mixed by gentle rotary movements. Tubes 1 and 2, precipitates in saliva secreted in response to chorda tympani stimulation; both types—almost solid lumps or a sticky mass with numerous threads adherent to the sides—are quite typical. Tube 3, precipitate in saliva secreted in response to intravenous injection of pilocarpine; similar to that in Tube 2. Tube 4, precipitate in saliva obtained on stimulation of the cervical sympathetic nerve, and Tube 5, in saliva secreted in response to intravenous administration of adrenaline; in both, protein material was precipitated in very small and light flocculi with no tendency to adhere to the sides.*

viscous solutions. The acetone precipitates obtained from chorda tympani saliva were fairly soluble in dilute alkalis, the solutions being extremely viscous. These precipitates could also be dissolved in distilled water and in 0.5*N* hydrochloric acid, but only after many hours of vigorous stirring; the resulting solutions were very viscous.

Thus on the basis of precipitability with trichloroacetic acid and with acetone it may be concluded that the parotid saliva and the parasympathetic and sympathetic submaxillary salivas have each a different protein.

A more detailed study was made of the general properties and elementary composition of the precipitates obtained by adding two volumes of acetone containing 3% of acetic acid to the submaxillary saliva secreted in response to stimulation of the chorda tympani or to injection of adrenaline. The corresponding precipitates will be referred to respectively as "Substance C" (chorda tympani saliva) and "Substance A" (adrenaline saliva).

The freshly secreted saliva was treated with acetone-acetic acid reagent immediately after collection of the sample; the precipitate was separated the following day by centrifuging, and washed twice with the same reagent diluted with one-third its volume of distilled water, twice with 95% alcohol, and finally with dry acetone and with ether. At this stage "Substance A"

was a very light, snow-white powder, readily soluble in distilled water, dilute alkalis (0.02*N* sodium hydroxide) or dilute acids (0.005*N* hydrochloric acid), the solutions being non-viscous. "Substance C" in appearance was a heavy, solid mass, which was very resistant to trituration in a mortar; in powder form it was completely soluble in 0.02*N* sodium hydroxide, but solution could be accomplished only after long and vigorous stirring. It was even more difficult to dissolve "Substance C" in distilled water. Its solubility in hydrochloric acid varied with the concentration; complete solution could be obtained only in concentrations slightly above 0.25*N*; once solution was complete, dilution with distilled water did not cause the characteristic precipitation described by Hammarsten (6) for his preparations of mucin from the submaxillary glands. All the solutions of "Substance C" (in distilled water, sodium hydroxide, or hydrochloric acid) were very viscous. Both "Substance C" and "Substance A" could be precipitated from these solutions in dilute hydrochloric acid with four volumes of absolute alcohol, "C" in the form of sticky masses (threads or lumps), "A" in the form of light flocculi. In both cases the precipitation was complete if traces of sodium chloride were added.

In this stage of preparation both "Substance A" and "Substance C" were obtained from many samples of saliva, and they showed quite consistent percentages of nitrogen content as determined by the Kjeldahl method, viz., for "Substance A", 8.8%, and for "Substance C", 8.2%. The reducing power, which was determined by the Hagedorn-Jensen method in the products of acid hydrolysis (3 hr. at 100° C. in *N* sulphuric acid), was identical for both preparations, being equivalent to a 33% glucose content.

An attempt was made to purify "Substance C" further, by the method of Hammarsten (6). For this purpose about 150 mg. of the substance was finely powdered in a mortar and dissolved in a minimal amount of water with the aid of dilute hydrochloric acid. Complete solution took place when the concentration of hydrochloric acid was increased to slightly above 0.25 *N*. When the solution was diluted with water no precipitation was observed. Therefore precipitation was effected with four volumes of alcohol and traces of sodium chloride. A heavy flocculent precipitate was formed almost instantaneously; on centrifuging it became a compact, sticky mass. This was separated by centrifuging and washed several times with alcohol in increasing concentrations, and finally with absolute alcohol, alcohol-ether (3 : 1), acetone and ether, and dried to a constant weight in a vacuum desiccator. In this form the product was just as resistant to pulverization as in previous stages. In finely powdered form it appeared as a pure white and remarkably heavy substance.

"Substance A" was purified in the same manner as "Substance C". The final product was a pure white substance which, in contrast to that from "Substance C", was very light and easily pulverizable.

The solubilities of both preparations were the same as in the first stage of purification.

The general protein colour reactions were positive both with "Substance A" and "Substance C". The proteins (0.2 and 0.4% solutions in 0.03*N* sodium hydroxide or in 0.03% hydrochloric acid) were not precipitable with tungstic or phosphotungstic acid any more than with hydrochloric, sulphuric, or acetic acid at the same pH. With phosphotungstic acid the solutions of both substances showed only a very slight opalescence at pH 1. With tungstic acid the solutions of "Substance A" remained quite clear throughout the range pH 7.0 to 1.0, whereas solutions of "Substance C" showed a slight turbidity at a faintly acid reaction to litmus and a small flocculent precipitate appeared at a point at which Congo paper turns distinctly blue; precipitation was never complete, however.

The viscosity (relative to that of water at 25°C.) was determined, by Ostwald's modification of Poiseuille's method (5, p. 71), in 0.4% solutions of Substances A and C in 0.003*N* sodium hydroxide and in 0.2% solutions in dilute hydrochloric acid. Acid solutions of both substances were prepared by dilution of the alkaline solutions with equal volumes of 0.017*N* hydrochloric acid. Both solutions remained clear. The results were as follows:

Solution	Water	0.4% alkaline		Water	0.2% acid	
		A	C		A	C
Time, sec.	80	156	372	79	82	90
Viscosity	—	1.95	4.65	—	1.04	1.14

Elementary analysis of the substances (nitrogen by the micro-Dumas method, total sulphur by the micro-Carius method) was carried out in the Laboratories of the Rockefeller Institute for Medical Research in New York, through the courtesy of Dr. P. A. Levene. Table II shows the results obtained, along with the analytical data for known preparations of mucin from the submaxillary glands for the sake of comparison.

A low percentage of nitrogen (from 8.20 to 8.89%) and a high reducing power after acid hydrolysis (equivalent to 33% of glucose) were consistently found in all the preparations isolated from both chorda tympani and adrenaline submaxillary saliva. These findings indicate that the proteins of the submaxillary saliva belong to the group of glucoproteins. This conclusion is in complete agreement with general opinion. The fact that the values were identical for both the nitrogen and the reducing power in the acetone precipitates before and after purification indicates that no other proteins are present in significant quantities in the submaxillary saliva of the cat. The fact that trichloroacetic acid does not cause any precipitation in submaxillary saliva suggests an even more definite conclusion, namely, that there are no other proteins, such as globulin or albumin, for example, in submaxillary saliva besides the two glucoproteins described above.

TABLE II
ELEMENTARY ANALYSIS OF VARIOUS MUCIN PREPARATIONS

	Substance A	Substance C	Scherer*	Obolenski*	Hammarsten*
C, %	—	—	52.00	52.30	48.80
H, %	—	—	6.90	7.20	6.80
N, %	8.76†	8.89†	12.80	11.90	13.30
	8.80‡	8.20‡			
S, %	1.83	2.43	—	—	0.84
Ash, %	0.85	1.25	—	—	—
Reducing power as % glucose after acid hydrolysis	32.80	32.90	—	—	—

* The data of Scherer, Obolenski, and Hammarsten are quoted from Table XIX, p. 126, "Hexosamines and mucoproteins", by P. A. Levene (Longmans, Green & Co., London, New York, etc., 1925).

† Dumas method.

‡ Kjeldahl method.

Thus, as shown above, "Substance A" (a protein isolated from the submaxillary saliva secreted in response to adrenaline administration) and "Substance C" (a protein isolated from the submaxillary saliva secreted in response to chorda tympani stimulation) show distinct differences in their general properties, especially with regard to the physical characteristics of the precipitates and the viscosity of their solutions. They also show significant differences in their elementary composition, particularly with regard to sulphur and nitrogen content (the latter as determined by the Kjeldahl method). Therefore these two substances must be regarded as two chemically distinct glucoproteins. This conclusion is corroborated by the recent observations of Langstroth, McRae, and Stavrazy (10), who found that the extinction coefficient for chorda tympani saliva, as determined by absorption spectrum measurements, and the protein-nitrogen values differ from those for adrenaline or sympathetic saliva.

Evidence has been accumulating lately that the chorda tympani and the sympathetic nerve supply different sets of cells in the submaxillary gland, the chorda tympani supplying the mucous cells and the sympathetic the serous cells (2-4, 10, 15, 17).

Thus all the experimental evidence so far available suggests that the mucous cells, which are under the control of the chorda tympani, secrete a specific glucoprotein different from the protein material secreted by the serous cells, which are under the control of the sympathetic nerve.

It is also of interest that both these proteins differ strikingly in their elementary composition from the preparations of mucin previously isolated from the submaxillary glands (6; 12, p. 126). This suggests the possibility that during the process of secretion the glandular proteins are transformed into the different types of proteins contained in the salivary secretion.

The Non-protein Nitrogen of the Submaxillary Saliva

As follows from the previous section, the proteins of the submaxillary saliva differ from those of the blood in respect of their precipitability by various reagents. From the analytical point of view, it is important that they are not precipitable either by tungstic acid or by trichloroacetic acid. Obviously none of the methods based upon the use of the above reagents are suitable for the quantitative determination of non-protein nitrogen in the submaxillary saliva. After having investigated a number of other protein precipitants we arrived at the conclusion that a very convenient and exact method of separating the protein from the non-protein fraction of the submaxillary saliva is precipitation of the saliva with two volumes of acetone, containing 3% of glacial acetic acid (described under Methods). The most convincing evidence of the completeness of fractionation by this method is the fact, repeatedly observed, that both the percentage of nitrogen in the acetone precipitate and the reducing power as determined after preliminary acid hydrolysis are constant and do not change even when the product is purified in the rather complicated manner described in the previous section. A further argument in favour of the procedure in question is that hundreds of protein nitrogen determinations by this method have been made by Langstroth, McRae, and Stavraký (10, 11), the results being checked by the spectrographic method, and it was found that they agreed well. Moreover the results thus obtained were sufficiently exact to permit of mathematical interpretation, which resulted in the development of a mathematical theory of protein secretion by the submaxillary glands (11).

Protein-free filtrates obtained by the above-described acetone method from several relatively large samples of submaxillary saliva (10.5 to 23 cc.) were analysed for their content of nitrogenous volatile bases, urea, creatine bodies, and amino-nitrogen. The results of these analyses are given in Tables III and IV; the former relates to the saliva secreted in response to chorda tympani stimulation and the latter to that obtained on administration of adrenaline.

While reserving fuller discussion of the above data for the following section, we should like to emphasize certain important observations here. Urea nitrogen is undoubtedly the main component of the non-protein fraction of the submaxillary saliva, forming from 50 to 86% of the total non-protein nitrogen. Both the absolute concentration of urea nitrogen in the saliva and the relative concentration with regard to total non-protein nitrogen are markedly higher in the adrenaline saliva and in the samples obtained by chorda tympani stimulation during the after-effect of adrenaline than the corresponding values in the chorda tympani saliva. Volatile nitrogenous bases were found to be always present in the submaxillary saliva in extremely low concentrations, namely from 0.4 to 0.7 mg. per cent nitrogen. These data are in complete agreement with the observations of Schmitz (19). Under the conditions of our experiments the variations to be found in Tables III and IV are entirely within the limits of experimental error. The amino-nitrogen

TABLE III
ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI

Experiment	Sample	Stim. chorda tympani, coil cm.	Secretion		Analytical data										Uniden- tified N, % of NPN	
			Dura- tion, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		
								Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %	Per cent of NPN	Mg. %		Per cent of NPN
No. 1, April 15 (1 gland only)	I	17	40	2.60	42.6	32.1	10.50	5.90	56.0	0.70	6.7	0.69	6.6	0.90	8.6	22.1
	II	12	30	4.40	17.5	9.1	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.90	10.7	17.5
	III	8	30	3.80	12.3	3.9	8.40	5.00	60.0	0.40	4.8	0.60	7.0	0.82	9.7	18.5
No. 2, May 8 (2 glands)	I	17	40	0.70	71.7	51.0	20.70	12.90	62.0	0.70	3.4	1.25	6.0	1.98	9.6	19.0
	II	12	30	3.05	42.6	25.8	16.80	11.40	68.0	0.60	3.6	1.19	7.0	1.70	10.0	11.4
	III	8	30	3.17	29.1	15.1	14.00	10.40	74.0	0.50	3.6	1.11	7.9	1.55	11.0	3.5
No. 3, March 10	I	12	18 hr.	1.50	—	—	9.65	6.63	68.7	0.56	5.8	0.80	8.3	0.35	3.7	13.5
Composite sample from several experiments		—	—	—	—	—	9.50	5.10	53.6	0.45	4.4	—	—	—	—	—

TABLE IV
ANALYSIS OF SUBMAXILLARY SALIVARY SECRETION EVOKED BY RHYTHMIC STIMULATION OF THE CHORDA TYMPANI OR ADMINISTRATION OF ADRENALINE

Experiment	Sample	Stimulation	Secretion		Analytical data										Uniden- tified N, Percent of NPN	
			Dura- tion, min.	Rate, cc. per 10 min.	Total N, mg. %	Protein N, mg. %	NPN, mg. %	Urea N		Volatile bases N		"Creatine bodies" N		Amino-N		
								Mg %	Percent of NPN	Mg. %	Percent of NPN	Percent Mg. %	Percent of NPN	Mg. %		Percent of NPN
No. 5 March 24	I	Chorda tympani, coil 16 cm.	40	2.90	41.4	29.7	11.7	6.20	53.0	0.50	4.3	0.56	5.0	1.3	11.1	26.6
	II	Adrenaline	80	0.50	35.8	8.7	27.1	17.50	64.6	0.50	1.8	1.43	5.3	2.2	8.1	20.2
	III	Chorda tympani, coil 12 cm.	60	1.90	47.0	19.2	27.8	21.50	77.3	0.50	1.8	1.34	4.8	1.7	6.1	10.0
No. 6 May 27	I	Chorda tympani, coil 15 cm	40	3.00	37.7	28.6	9.1	5.04	55.4	0.70	7.7	0.50	5.5	0.4	4.4	27.0
	II	Adrenaline	60	0.45	61.1	38.7	22.4	13.45	60.5	0.70	3.1	1.50	7.0	0.9	4.0	25.4
	III	Chorda tympani, coil 7 cm	30	2.66	58.8	36.2	22.6	16.80	74.4	0.70	3.1	1.60	7.1	0.7	3.1	12.3
Composite sample from several ex- periments		Adrenaline	—	—	—	—	25.8	19.00	73.6	0.52	2.0	—	—	—	—	—

values are also low, ranging from 0.4 mg. per cent to 2.2 mg. per cent. In this respect our data agree with the observations of Updegraff and Lewis (20).

In the tables the figures arbitrarily given as representing creatine + creatinine nitrogen do not represent only these substances, since the development of the colour in most cases was attained almost at once on adding alkali to the mixture of protein-free filtrate and picric acid, and furthermore the colour did not fade as quickly as in the solutions of creatinine having the same maximum colour. The concentrations of apparent creatine + creatinine were found to be from 0.5 to 0.6 mg. per cent in typical chorda tympani saliva, and were considerably greater in the samples obtained in response to injection of adrenaline, i.e., from 1.43 to 1.5 mg. per cent, and remained high in the samples obtained on chorda tympani stimulation during the after-effect of adrenaline (viz., 1.34 to 1.6 mg. per cent).

Discussion

Considerable variations were observed in the concentrations of the various nitrogenous constituents of submaxillary saliva, depending on the conditions of stimulation. A sharp progressive fall in the concentration of protein nitrogen in the successive samples of saliva obtained by rhythmic stimulation of the chorda tympani (cf. Table III) was regularly observed, which merely confirms facts established long ago. Intravenous administration of adrenaline in massive doses interposed between two periods of chorda tympani stimulation (Table IV) resulted in an increase in the concentration of total nitrogen in the chorda tympani saliva obtained after adrenaline administration. Whereas the concentration of non-protein nitrogen in this type of saliva was always greater, the percentage of protein nitrogen varied from one experiment to another. Thus, e.g., in the experiment of March 24, it was lower in the chorda tympani saliva obtained after adrenaline administration than in that obtained before, while in the experiment of May 27 it was definitely higher. These variations might be due partly to the degree of vasoconstriction produced by massive doses of adrenaline and the necessity of applying a stronger electric current to the nerve in order to obtain an adequate volume of saliva. Furthermore Langstroth, McRae, and Stavraky (10) consider that prolonged vasoconstriction produced by adrenaline administration may be partly responsible for the increased concentration of organic colloidal material in cat's submaxillary saliva obtained by subsequent stimulation of the chorda tympani.

Another conclusion which may be drawn from these experiments concerning the protein material in the submaxillary saliva is that the total output of protein material in the submaxillary saliva in response to adrenaline stimulation is not influenced by the previous activity induced in the gland by chorda tympani stimulation, but presumably depends on the store of the parent substance in the form of granule material in the serous cells. This is evident from the fact that in two experiments (Nos. 5 and 6) the rate and the duration of secretion obtained on administration of adrenaline were practically identical

(rate 0.5 and 0.45 cc. in 10 min., duration 80 and 60 min. respectively), but the concentration of protein nitrogen differed markedly in the two samples (being 8.7 and 38.7 mg. per cent). It is important to note in this connection that the preliminary secretions of the gland in response to chorda tympani stimulation were almost similar in amount in these experiments (viz., 2.9 and 3.0 cc.), that they were obtained over the same period of time (40 min.) and that they contained practically the same amount of protein (29.7 and 28.6 mg. per cent of protein nitrogen). These facts support the theory that parasympathetic and sympathetic stimulation respectively act on different cytological elements of the submaxillary gland.

Our data on non-protein nitrogen and its various fractions permit some conclusions to be drawn concerning changes in the "permeability" of the submaxillary glands to these substances under various conditions of stimulation. It was found practical for the purpose to classify the various fractions of non-protein nitrogen into three main groups:

- (1) Urea nitrogen (represented in Figs. 2 and 3 by the symbol "UrN").
- (2) "Creatine bodies fraction" + "volatile bases fraction" + amino-nitrogen ("RN").
- (3) Unidentified nitrogen ("XN").

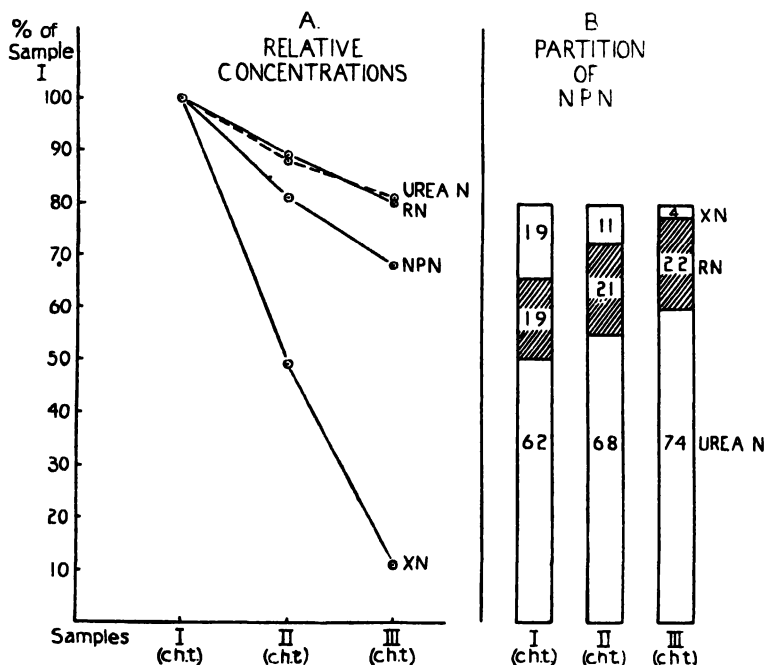


FIG. 2. Experiment 2. Influence of chorda tympani stimulation on the non-protein nitrogen of submaxillary saliva.

Samples I, II, and III are successive samples. (For full data see Table III.) Section A, values expressed as percentages of values for the first sample. Section B, values for three main fractions of non-protein nitrogen expressed as percentages of non-protein nitrogen. NPN, non-protein nitrogen; RN, combined nitrogen of the "creatine bodies" + volatile bases + amino-nitrogen; XN, unidentified nitrogen.

Although in different animals the initial values both for total non-protein nitrogen and for its fractions varied, each experiment provided convincing evidence that the concentration of certain moieties in the saliva underwent marked changes under various experimental conditions. The most probable explanation of this phenomenon is that the changes in the composition of the saliva occurred as a result of altered permeability of the cellular membranes.

Fig. 2 represents the results of two experiments in which the chorda tympani was stimulated. Section A shows changes in the concentrations of the three main fractions, expressed as percentages of the values for the first sample; Section B shows the relative concentrations of the same three fractions as percentages of the total non-protein nitrogen. In both experiments qualitatively identical results were obtained: the concentrations of total non-protein nitrogen and of each of the main fractions are lower in each successive sample; the relative concentration of "unidentified nitrogen" decreases in the successive samples to a much greater extent than that of the other fractions. Section B shows how these processes affect the partition of non-protein nitrogen in each consecutive sample. It will be seen that, as the glands secrete under the influence of the chorda tympani, an increasingly greater part of the non-protein nitrogen is formed by urea, while the fraction of the creatine bodies + volatile bases remains virtually stationary and the percentage of "unidentified nitrogen" falls considerably (especially in Experiment 2). The obvious conclusion is that continuous stimulation of the chorda tympani for several hours causes an appreciable decrease in the glandular permeability, which though it affects but slightly the passage of the small molecules such as urea (and perhaps "RN"), inhibits considerably the passage of the larger molecules such as "unidentified nitrogenous substances". This conclusion is in agreement with the results of Langstroth, McRae, and Stavraký (10), who observed a gradual decrease in the concentrations of the total anions and non-protein nitrogen in the saliva secreted under chorda tympani stimulation and ascribed this phenomenon to decrease of the glandular permeability.

Fig. 3 shows the results of Experiments 5 and 6, in which between two periods of prolonged chorda tympani stimulation (Samples I and III), the submaxillary gland was subjected for 80 and 60 min. respectively to stimulation by repeated injections of massive doses of adrenaline (Sample II). In both experiments the absolute concentration of every fraction of non-protein nitrogen was increased in the samples of adrenaline saliva, but in various degrees. In Experiments 5 and 6 respectively the concentration of non-protein nitrogen rose to 232 and 240%, that of urea nitrogen to 282 and 267%, while "RN" was 175 and 194%, and unidentified nitrogen 168 and 238%, of the initial values. After the administration of adrenaline was discontinued and chorda tympani stimulation was recommenced, the urea nitrogen continued to rise, reaching 347 and 333%, the "RN" fell slightly to 150 and 188%, while the unidentified nitrogen returned practically to the

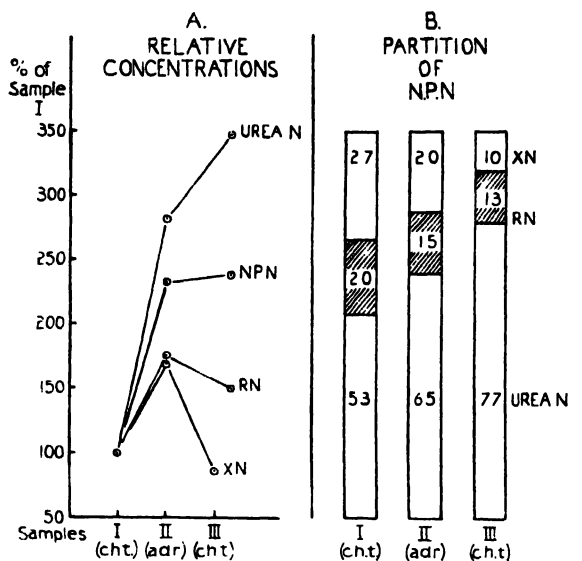


FIG. 3. Experiment 5. Effect of adrenaline on the non-protein nitrogen of submaxillary saliva.

Samples I (ch. t.), II (adr.), III (ch. t.) were obtained by chorda tympani stimulation, adrenaline administration, and chorda tympani stimulation immediately after discontinuing adrenaline. (See also Table IV.) Explanation of symbols as in Fig. 2.

initial values, being 85 and 114%, and the non-protein nitrogen remained stationary. Thus the administration of adrenaline increased the permeability of the gland to all fractions of non-protein nitrogen and especially to the passage of urea. The greater permeability to urea persisted or even continued to increase further for a considerable time after the administration of adrenaline was stopped and chorda tympani stimulation substituted, but the permeability to unidentified nitrogen soon returned to normal. The final result of all these phenomena was a steady and marked increase in the percentage of urea nitrogen and progressive decrease in the percentage of unidentified non-protein nitrogen, with the percentage of "RN" remaining practically stationary. The above-reported facts supplement the results of Langstroth, McRae, and Stavrazy (10), who found that samples of adrenaline saliva are richer in inorganic salts, and samples of chorda tympani saliva obtained after adrenaline administration are richer in inorganic salts and glucose than samples of chorda saliva obtained before adrenaline administration. They attributed these results to the increased permeability of the glandular membrane which persists for several hours after adrenaline administration.

Acknowledgment

Our thanks are due to Professor B. P. Babkin for the helpful advice and criticism that he has given us during the course of this work.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

JULY, 1940

NUMBER 7

CANADIAN WILTSHIRE BACON

X. DISTRIBUTION OF CHLORIDE¹

BY W. H. WHITE² AND W. H. COOK²

Abstract

Highly significant differences in chloride content were observed at different positions across the ham of Wiltshire sides, the portion next to the tank pickle during cure usually containing the most chloride. Of the various factors studied, the number of injections used for pumping a side, and the age from cure, were the only ones found to affect the chloride distribution. Greater uniformity was obtained by increasing the number of injections, and with increasing age from cure. Within the range used in practice for curing Wiltshire sides, the chloride content of the pickles or the number of days in cure was not related to the chloride distribution. An equation is given relating the uniformity of chloride distribution to the number of injections used per side in pumping, and the number of days from the end of cure. This equation indicates that, on the average, the variations in the chloride content within the ham of a Wiltshire side will be reduced to the same magnitude as the variations between sides cured in the same plant, after holding at 1.1° C. for periods of about 12, 26, and 30 days, for sides receiving 27, 8, and 0 injections, respectively.

Introduction

Curing of bacon involves not only the addition of a sufficient quantity of curing salts, but also their distribution throughout the meat. The period of maturation, following removal of the bacon from cure, is believed to improve the quality of the product, particularly with respect to colour uniformity and stability, and the development of the typical bacon flavour. Because of the relatively short curing periods employed in the preparation of Wiltshire bacon, it seems probable that the distribution of salts takes place mainly after removal from cure. Presumably such distribution must reach a certain level of uniformity before the desirable changes involved in maturation can occur. The apparent importance of a uniform salt distribution, both from its direct effect on palatability and its possible indirect effect on maturation, led to a study of this subject on factory-cured Wiltshire bacon.

The content of curing salts and properties of Wiltshire bacon obtained from different Canadian packing plants, and the effect of storage and smoking on these quantities, have been reported in earlier papers of this series (5, 6). The present study was confined to the distribution of chloride throughout the ham

¹ Manuscript received March 21, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 43 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 921.

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at the different samplings, with a few additional determinations on the content and distribution of chloride, nitrate, and nitrite, in pumped and unpumped sides.

Since the sides obtained from different plants varied in age from two to 11 days from the end of cure when the first analyses were made, it is obvious that the results do not give a measure of the rate of salt penetration during cure. In commercial practice, moreover, pickle is injected into Wiltshire sides at a number of points before they are placed in the curing tank. This practice, termed pumping, doubtless tends to increase the total salt content and to improve the uniformity of salt distribution, but the variations between the methods employed in different plants could easily mask the effect of differential rates of salt penetration in different carcasses. Factors involved in pumping include the amount of pickle injected, and the position and number of stitches used. Of these quantities, only the effect of the number of stitches could be determined, since the position of the stitches cannot be described in quantitative terms, while the amount of pump pickle added to the sides in question was unknown. It appears, however, that the quantity of pickle retained by the sides is a function of the number of stitches employed (4).

Material, Sampling, and Methods

A description of the sides used in this study, the method of transfer from the plants to the laboratory, and the conditions of storage between samplings, has been reported in the first paper of this series (7). The samples were obtained in the conventional manner (1) from a central strip, about $1\frac{1}{2}$ in. wide, taken from the slice of ham removed for analysis at each sampling (7). These strips included the cross section of the femur, since certain practical operators attach considerable importance to the salt concentration of the meat in the vicinity of the bone. There is also some evidence (3) that the adductor and vastus intermedius muscles do not absorb salt readily. Each strip was divided into three, instead of the five or more sections usually employed in studies of this sort. This practice was followed since the hams on Wiltshire sides are rather small and, being compressible, tend to vary somewhat in thickness, depending on the conditions and method of baling and transport. The position from which each of the samples was taken is shown in Fig. 1. The material from Position 1, on the inside of the ham, was adjacent to the tank pickle during cure; that from Position 2 represented the middle of the ham, surrounding the bone; while Position 3 represented the meat adjacent to the layer of fat and skin, through which little salt is likely to penetrate during cure.

The lean meat was separated from the bone, fat, and connective tissue, and each portion thoroughly mixed and ground by passing it through a food chopper. The samples were then frozen and stored at -29°C. , until required for analysis. The chloride content was determined according to the official method of the A.O.A.C. (9, p. 354), in which the sample is ashed, leached with hot water, and the chloride content of this extract found by the Volhard



FIG. 1. *Portions of Wiltshire hams used for determining distribution of chloride.*

procedure. Statistical methods (10) were employed for interpreting the results.

The entire group of 44 sides were analysed at the first and second samplings; i.e., when received at the laboratory, and again after 10 days' storage at 1.1° C. Only 16 of these sides were analysed for chloride distribution at the third sampling, i.e., after smoking. While in subsequent tables the results for the entire group are considered together for the first two samplings, those for the 16 sides analysed three times are also considered separately, in order to provide comparable results over all three samplings.

Mean Chloride Content by Positions and Samplings

The mean age and sodium chloride content by positions and samplings over all sides are given in Table I. The chloride content of the slice of ham from which the chloride distribution samples were taken has already been reported (6). The table is divided into two main sections, the first giving the results for the 44 sides analysed twice and the second the results of the 16 sides analysed three times.

Although the mean age of the entire group of sides from cure was about seven days at the time of the first sampling, it is evident that the sodium chloride content at Position 1, next to the tank pickle, was much higher than at Positions 2 and 3. This gradient still existed, but was much smaller, at the second sampling when the sides were about 18 days old. The group of 16 sides analysed three times indicated essentially the same behaviour at the first two samplings, and also showed that the chloride is quite uniformly distributed after smoking, when the mean age for the group was about 24 days.

The results reported in the last column represent the best estimate of the true chloride content of the hams, since these figures (6) were obtained by grinding and sampling a piece of each ham several times as large as that of the entire strip used in the salt distribution studies. Other investigators (8) have observed that this central-strip sampling procedure does not represent

TABLE I
MEAN SODIUM CHLORIDE CONTENT OF SIDES BY POSITION AND SAMPLING

Sampling	Mean age of sides from cure, days	Position	Mean sodium chloride content		
			By position, %	By sampling, %	From previous analyses (6), %
For all 44 sides—sampled twice					
First	7.4	1	6.42	4.30	3.43
		2	3.45		
		3	3.03		
Second	17.8	1	4.49	3.91	4.42
		2	3.78		
		3	3.46		
For 16 sides—sampled three times					
First	9.9	1	6.87	4.34	3.19
		2	3.47		
		3	2.69		
Second	21.4	1	4.46	3.84	4.19
		2	3.73		
		3	3.33		
Third	24.4	1	4.06	3.99	—
		2	3.90		
		3	4.01		

the true salt content of the ham. The present results indicate that the mean chloride content over all three positions in this central strip apparently overestimates the true chloride content at the first sampling and underestimates it at the second. In more specific terms the chloride content at Position 1 was much above the average at the first sampling, while that at Positions 2 and 3 was still below the average at the second sampling. From this it appears that the positions chosen represent not only those likely to have the highest chloride content initially, but also those that are the last to be affected by the movement of chloride throughout the ham.

The significance of the observed differences in the chloride content between positions at the different samplings was determined by an analysis of the variance for each sampling, into portions attributable to: between positions; between sides from different plants; between sides from the same plant; the interaction positions \times plants; and sampling and analytical error. The results, which appear in Table II, show that the difference between sides from different plants is usually significantly greater than the differences between sides from the same plant, and that this in turn usually exceeds the sampling and analytical errors. This merely confirms the findings reported

TABLE II
ANALYSIS OF VARIANCE OF SODIUM CHLORIDE CONTENT AT DIFFERENT SAMPLINGS

Variance attributable to	Sampling					
	First		Second		Third	
	D.F.	Mean sq.	D.F.	Mean sq.	D.F.	Mean sq.
For all 44 sides—sampled twice						
Between positions	2	149.926**	2	12.206**	—	—
Between plants	21	4.148	21	3.320**	—	—
Between sides	22	2.202**	22	0.797**	—	—
Differential effect, position × plants	42	1.977**	42	0.433**	—	—
Sampling and analytical error	44	0.674	44	0.177	—	—
For 16 sides sampled for salt distribution—three times						
Between positions	2	79.186**	2	5.262**	2	0.102
Between plants	7	8.129**	7	5.227**	7	7.329**
Between sides	8	0.798**	8	0.583	8	0.844
Differential effect, position × plants	14	1.232**	14	0.343	14	0.250
Sampling and analytical error	16	0.175	16	0.287	16	0.434

* Indicates 5% level of significance.

** Indicates 1% level of significance.

in an earlier paper (6). The fact that these differences were sometimes not significant at certain samplings in the present study does not indicate disagreement with the earlier results, since they dealt with the average effect over all sides and samplings.

The present study is concerned primarily with the variance between positions, since this quantity reflects the variations in chloride distribution. It is evident that the observed differences in the chloride content at different positions, as indicated by the results in Table I, are statistically significant at the first two samplings. Since the mean square between positions decreases between successive samplings, it is evident that the chloride becomes more uniformly distributed during the maturation period. In the group of sides sampled after smoking, the difference between positions is not significant, indicating the absence of a systematic gradient in chloride concentration.

The interaction position × plants was also significant at both samplings for the entire lot of 44 sides. This indicates that there was not a uniform gradient in chloride concentration from one position to another in the product of all plants. It is possible that this could be attributed to the different pumping practices, etc., followed in different plants. Since the sides from the different plants varied in age from two to 11 days from cure at the time

of the first sampling, it may be that the different gradients merely reflect the effect of ageing.

Chloride Distribution in Relation to Curing Practice

Before attempting to establish a quantitative relation between the chloride distribution and the age of the sides, some consideration had to be given to the effect of variations in the curing practices, in order that these factors could be taken into account. The standard deviation between the positions for both sides received from each plant was used as an estimate of chloride distribution. Although the standard deviations between all three positions were used initially, it was found, as the computations proceeded, that the difference between Positions 1 and 3 was an equally sensitive measure of the variations in chloride content. The standard deviation or difference between the positions observed for all sides at the first sampling was correlated with the known curing factors, as outlined in the first part of Table III. The results of the second sampling were not included, since the influence of age tends to minimize any difference attributable to the various curing practices.

Examination of these correlation coefficients shows that only the coefficient relating the standard deviation between positions and the number of stitches used per side attains the 5% level of significance. In fact, the other coefficients relating quantities involving the number of stitches per side, although not significant, were much larger than the correlations between the standard deviation and other quantities, and probably would have been significant had the sides been analysed immediately after cure. It is therefore concluded that chloride distribution is influenced more by the number of stitches used in pumping than by the observed variations in the chloride content of either of the pickles or by the number of days in cure.

In an earlier paper (6), it was shown that the number of stitches injected per side was the only known curing practice that was correlated with the over-all chloride content of bacon. Since the chloride content at Position 1 should be affected primarily by the tank curing practice, and that at Positions 2 and 3 by the pumping practice, correlation coefficients were computed between the chloride contents at these positions, for the first sampling, and the known properties of the pumping and tank curing practices. The results obtained are shown in the second part of Table III. Although none of the correlation coefficients are significant, their magnitude suggests that the number of stitches per side is the most influential factor of those studied in determining the chloride content at Position 2. It appears, therefore, that within the limits used in practice, similar amounts of chloride are taken up from the tank pickle regardless of its concentration or period in cure. In these circumstances, pumping the sides would be expected not only to reduce the variation in chloride content between the outside and the centre positions, but also to increase the total amount in the bacon. Nevertheless, correlation coefficients failed to demonstrate a definite relation between chloride content and chloride distribution.

TABLE III

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE DISTRIBUTION
AND CERTAIN CURING PRACTICES

Quantities correlated	D.F.	
Sodium chloride distribution in relation to curing practice		
Standard deviation of sodium chloride content at all 3 positions with:		
No. of stitches per side	20	-0.42*
No. of stitches per side \times sodium chloride in pump pickle	20	-0.38
Difference in sodium chloride content between Positions 1 and 3 with:		
No. of stitches per side	20	-0.42*
Days in cure	20	-0.14
Sodium chloride in tank pickle	20	+0.01
No. of stitches per side \times sodium chloride in pump pickle	20	-0.38
No. of stitches per side, independent of days in cure	19	-0.41
No. of days in cure, independent of number of stitches per side	19	-0.08
Sodium chloride content in relation to curing practice		
Sodium chloride content of meat at Position No. 1 with:		
Days in cure	19	+0.14
Sodium chloride in tank pickle	19	+0.01
Sodium chloride in tank pickle \times days in cure	19	+0.14
Sodium chloride content of meat at Position No. 2 with:		
No. of stitches per side	20	+0.37
No. of stitches per side \times sodium chloride in pump pickle	19	+0.34
Sodium chloride content of meat at Position No. 3 with:		
No. of stitches per side \times sodium chloride in pump pickle	19	+0.07
Relation between distribution and content of sodium chloride		
Standard deviation in sodium chloride content at all 3 positions with mean chloride content of all 3 positions for:		
First sampling by plants	20	-0.18
First sampling by sides	42	-0.11
Second sampling by plants	20	-0.36
Second sampling by sides	42	-0.29

* Indicates 5% level of significance.

Comparison of Pumped and Unpumped Sides

The results presented in this and earlier papers (5, 6) have indicated the importance of the method of pumping, in determining the concentration of curing salts in bacon. To confirm this a few preliminary experiments were made on the composition of pumped and unpumped sides cured simultaneously in the same tank in a packing plant. Four pumped and two unpumped sides were sampled and analysed, by the methods previously described (11), immediately on removal from cure and again 17 days later. The mean com-

position of each group of sides, with respect to sodium chloride, nitrate, and nitrite, appears in the first part of Table IV, and the chloride distribution, as shown by the sodium chloride content at the different positions, in the second part. Statistical methods were not applied to these few results, but since the different sides in each group varied considerably, only relatively large differences can be considered significant.

TABLE IV
COMPOSITION OF, AND CHLORIDE DISTRIBUTION IN, PUMPED AND UNPUMPED SIDES

Constituent	Pumped		Unpumped	
	1st sampling	2nd sampling	1st sampling	2nd sampling
Constituent—				
Sodium chloride, %	2.61	3.68	0.56	1.93
Sodium nitrate, %	0.16	0.16	0.008	0.028
Sodium nitrite, p.p.m.	17	34	0.8	27
Sodium chloride distribution				
Position—				
1	4.92	2.96	3.15	2.28
2	3.08	2.91	0.82	1.26
3	3.41	3.48	0.46	1.05

It is evident that the slice of ham analysed at the first sampling contained less of all three salts than at the second sampling. In general, this apparent increase in the content of the curing salts must be attributed to differences between positions rather than a change with time (6). Nevertheless, the proportionately greater increase in the nitrite content of the unpumped sides between samplings suggested a real change during storage. This confirms an earlier finding (6) that nitrite is produced in sides having a low nitrite content initially.

Comparison of the values obtained at the second sampling, when a reasonably uniform distribution of the salts has occurred, indicates that pumping contributes about half the chloride and 80% of the nitrate found in the bacon. The chloride content at the three positions for the second samplings suggests a relatively uniform distribution throughout the hams of the pumped sides, while the unpumped sides still had about twice as much chloride at the position adjacent to the tank pickle, as at the central positions. The importance of pumping in determining both the concentration of the salts and their distribution in the sides is apparent.

Effect of Pumping and Ageing

It was concluded from the results already presented that the number of stitches used for pumping and the age from cure were the principal factors affecting the distribution of salts in hams of Wiltshire sides. In order to obtain

a quantitative estimate of the effect of these two factors, a partial regression equation was computed for the standard deviation between positions on number of stitches used in pumping and the number of days from cure over all samplings. This equation was found to be:

$$Y = 4.64 - 0.074 S - 0.126 N$$

where

Y = standard deviation between salt content at different positions,

S = number of stitches used per side

and

N = number of days from cure.

Although this equation shows the extent to which the variations in chloride content between different positions decrease with increase in number of stitches per side and days from cure, it is also important to determine the amount of the total variance accounted for in this way. The mean square for the observed variations in Y (standard deviation between positions) was found to be 1.42, and after subtracting the variance accounted for by the above equation, the residual mean square was 0.70. This shows that variations in pumping practice and age of the sides accounted for about half of the observed variance.

From a practical standpoint, there is no apparent advantage in reducing the variations in salt content within an individual side to less than those between different sides produced under the same conditions in the same plant. Reference to Table II shows that the mean square between sides over all samples at the second sampling was 0.80.

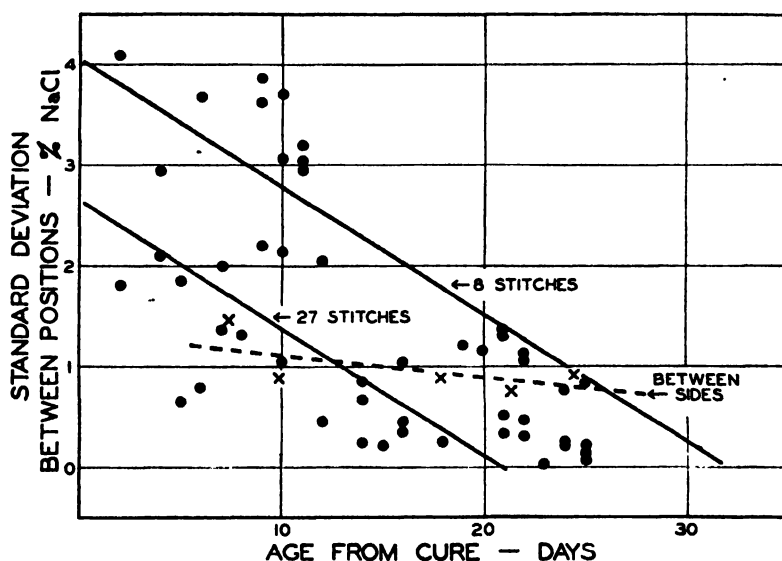


FIG. 2. Effect of number of stitches used in pumping, and the age of the side on the distribution of chloride.

Using this criterion, it is possible to predict the time required, following various pumping practices, to obtain a satisfactory chloride distribution throughout the ham of a Wiltshire side. In order to do this the equation given earlier was used to construct Fig. 2, in which the estimated standard deviation between positions is plotted against the age of the sides from cure. The upper line represents the results with the minimum (eight), and the lower, with the maximum (i.e., 27) number of stitches used for pumping the sides studied in this investigation. The experimental points, representing the mean value for the two sides received from each plant, are plotted for comparison. The crosses and broken line indicate the level of the standard deviation between sides, computed from the mean square given in Table II, for sides of various ages from cure. The time required for the standard deviation between positions to fall to this level represents that required to obtain a satisfactory chloride distribution. The figure suggests that this level could be attained in about 12 and 26 days, respectively, for sides receiving the maximum and minimum number of stitches ordinarily used in pumping. Since Canadian sides never arrive in England in less than 12, and on the average 18, days after removal from cure, it appears that they must be satisfactory from the standpoint of chloride distribution before they reach the consumer.

Discussion

Considerable work has been done on the rate of salt penetration into meat, and its subsequent distribution within the cured product. An extensive review of the literature on this subject is unnecessary, but it is of interest to consider the findings of certain investigators in relation to those obtained in this study. Reduced to essentials, the present results show that the number of stitches used in pumping and the age of the sides from cure were the only factors of those studied found to affect the chloride distribution in Wiltshire bacon as manufactured by current methods.

Banfield and Callow (2) have shown that the decrease in electrical resistance during cure was less marked in unpumped than in pumped sides. This implies that the unpumped sides contained less salt, a result confirmed by analysis after maturation, although the small difference observed for tank cured sides is doubtless of little practical consequence. These investigators used the backs, and since the lean portion at this point is smaller than that of the ham, a more uniform penetration and distribution of the chloride is to be expected, and the effect of pumping consequently less marked.

Besley and Hiner (3) have shown that the various muscles in unpumped lamb legs absorb salt differentially, and claim that even larger differences occur between the different muscles in hams. The results of the present study suggest that where a reasonably satisfactory pumping practice is used, with respect to both the number and position of the injections, the differential absorption of chloride by different muscles would have little effect on the chloride distribution. They also found that the number of days in cure was the principal factor affecting the chloride content and distribution between different

muscles. Since these lamb legs were both unpumped and cured for a period far in excess of that generally used for curing Wiltshire bacon, these findings do not appear to contradict those obtained in these investigations (3).

Miller and Ziegler (8) studied the chloride distribution in hams, using sampling and statistical methods comparable to those used in this study. The hams, however, were not pumped and the length of the curing period was considerably longer than that used for Wiltshire sides. Their results indicate that following a brine-curing period of 30 days, a holding period of another 30 days at 37° F. was required to reduce the coefficient of variability between positions to about 20% or less. This variability corresponds to a standard deviation of about 0.80% when interpreted in terms of the mean sodium chloride content, expressed on a "wet" basis, as observed in these studies and reported in Table I and previously (6). Using the equation given earlier, with $S = 0$, since no pickle was injected, and $Y = 0.8$, a value of about 30 is obtained for N . It is indeed surprising that the results of these two independent investigations agree so closely, considering that they represent work with pumped sides and unpumped hams, cured for widely different periods, and subsequently stored at somewhat different temperatures.

Acknowledgments

The authors wish again to express thanks to the firms and individuals mentioned in the first paper of this series, and particularly to Messrs. A. E. Chadderton and G. N. Seed, laboratory assistants, who were responsible for most of the sampling, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on the statistical treatment of the results.

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CANADIAN WILTSHIRE BACON

XI. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT¹By W. H. WHITE², W. H. COOK², AND C. A. WINKLER³

Abstract

Small cuts of matured Wiltshire bacon were held at temperatures of 20, 40, 50, 60, 70, and 80° C. for periods of 5, 10, 20, and 40 hours. When the nitrite content after treatment was plotted against the temperature, the results showed that the highest nitrite contents were obtained at 40° C., the nitrite content decreased logarithmically between 40 and 80° C., and the slope of these curves increased with increasing periods of treatment. The curves representing the different periods of treatment intersect in the region of 55° C., where the nitrite content was approximately normal. It appears, therefore, that an increase in nitrite content above the normal level occurred at temperatures below 55° C. and a decrease at higher temperatures. This decrease in nitrite nitrogen above 55° C. may be attributed to direct loss, oxidation, or reaction with the constituents of the meat. The increase in nitrite observed at temperatures below 55° C. suggests that time and temperature were not alone responsible for the observed changes, since comparable increases in nitrite were not observed in commercial cuts. It may be that the proportionately greater surface exposed to the air in these small samples may have had some effect, such as an enhanced aerobic bacterial action.

Introduction

The characteristic colour of bacon is attributed primarily to the combination of nitrite with the muscle haemoglobin to form nitrosohaemoglobin. This compound is believed to be converted to a more stable form, termed nitrosohaemochromogen, when the meat is cooked, and this change may also occur to some extent during smoking at higher temperatures, i.e., above about 55° C. In earlier papers (7, 8) of this series, it has been shown that the pH and nitrite content of the bacon affected colour and colour stability. It may be that these influential factors act either by determining the rate or extent of the reaction producing nitrosohaemoglobin, or by an independent effect on the colour of the bacon. The nitrite content of bacon may not reflect its nitrosohaemoglobin content, but as a factor affecting colour it deserves consideration. It has also been shown (3, 6) that the nitrite content of certain sides, through bacterial action or otherwise, may increase during holding after cure, and decrease slightly during smoking at about 45° C. (3).

An investigation was, therefore, undertaken to determine the effect of heating Wiltshire bacon, for various periods at different temperatures, on its nitrite content and colour. This paper deals with the changes in nitrite content.

¹ Manuscript received March 21, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 44 of the Canadian Committee on the Storage and Transport of Food, and as N.R.C. No. 922.

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Material, Design, and Method

Since the earlier studies (3) showed that different sides, treated in the same manner, differed significantly in nitrite content, it seemed desirable to have different sides cured in several different plants represented in the experiment. The available material consisted of "backs", cut from two sides (from different pigs) cured in each of eight different packing plants. These sides represented bacon of various ages, having been removed from cure and held at temperatures of 1 to 3° C. for periods of from 23 to 25 days before being sampled for these studies.

The experiment was arranged in a factorial design (4), involving: 4 times of treatment, namely, 5, 10, 20, and 40 hr.; 6 temperatures, namely 20, 40, 50, 60, 70, and 80° C.; and 4 replications of each treatment. The 96 pieces required were obtained as follows: The 16 backs were randomized over the 4 replicates and 4 times of treatment; each back was then divided into 6 approximately equal parts of about 100 to 200 gm. each, and these pieces were randomized among the 6 temperatures within times. It is obvious that this design yields more precise information on the effect of temperature than of time, since the significance of the observed differences with temperature is tested by comparison with the analytical and sampling error within sides, while the differences between times of treatment must be tested by comparison with the difference between sides. Within the limitations of available material, time, and facilities, it seemed more desirable to obtain precise information on the effect of temperature.

The samples were first trimmed free of the cut portions of the ribs and the layer of back fat. The individual pieces were weighed, wrapped in lightly waxed glassine, and then in kraft paper. The individual packages to be heated at a given temperature were wrapped into a single package. These operations were performed in a laboratory at about 25° C. The samples were placed in ovens at the required temperatures, removed after the required period of time, and weighed. The surface layers were trimmed off, suitable samples taken for colour measurements, and the remainder, trimmed free of fat and connective tissue, was ground and mixed by several passages through a food chopper, bottled, and frozen at -29° C. The samples were thawed as required, analysed by the method previously described (5), and the results treated by statistical methods (4).

Results

The loss of weight during heating was a function of the time and temperature of treatment. At the higher temperatures these losses represented not only a loss of moisture but also of a certain amount of fat that melted and was absorbed by, or lost from, the paper wrapping. These figures were used for computing the amount of nitrite in terms of the original sample weight, but otherwise are of little significance in the present study.

The mean nitrite content of the four samples treated under each condition is reported in Table I. In considering these results, it must be remembered that there was considerable variation between the individual pieces from different sides treated in the same way. This, together with the fact that the nitrite content after the different treatments also varied widely, made it necessary to employ statistical methods to test the significance of the observed differences.

TABLE I
MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS
(As p.p.m. in initial weight)

Period of heat treatment, hr.	Temperature of heat treatment, °C.					
	20*	40	50	60	70	80
5	25.81	22.16	19.73	16.94	20.16	14.38
10	29.21	41.88	41.40	15.02	8.62	5.49
20	179.52	191.25	51.35	10.20	5.76	2.36
40	—	404.75	33.95	9.41	3.82	1.32

* Samples treated at this temperature considered separately in subsequent statistical analysis.

The values reported in Table I show that the samples varied in nitrite content from about one to over 400 p.p.m. The deviations of the individual values from the reported means were approximately proportional to the mean. Since results exhibiting a variability of this magnitude cannot be used directly for an analysis of variance (2), they were first converted into logarithms of the nitrite content in parts per million before the statistical computations were undertaken. All numerical values subsequently reported are on this basis.

The samples held at 20° C. were used to determine the changes in nitrite content that occur at room temperature. Unfortunately, the samples that were to be held for 40 hr. at this temperature were removed and ground after 20 hr., so that 8 samples were treated for 20 hr. at this temperature but none for 40 hr. This loss was not serious, however, since it seemed desirable, in making the statistical analyses, to treat the results obtained at 20° C. separately from those obtained at higher temperatures.

The results of an analysis of variance are reported in Table II. It is evident that the difference between the nitrite content of the samples held at 20° C. is highly significant. This shows that the increase in nitrite content, as shown in Table I, exceeds the variations observed between different sides, even within a 20-hr. period. The remaining samples, treated at higher temperatures, were considered together, and for these the average change in nitrite content with time is not significantly greater than the differences between the different sides that were treated similarly, but is significantly greater than the sampling and analytical error within sides. From

TABLE II
ANALYSIS OF VARIANCE. EFFECT OF HEAT TREATMENT ON NITRITE CONTENT
OF BACON

(Values in terms of logarithms of nitrite content in p.p.m.)

Variance attributable to	Degrees of freedom	Mean square
Samples heated at 20° C.		
Between times of treatment	2	1.426**
Between sides within times	13	0.124
Samples heated at 40 to 80° C.		
Between times of treatment	3	0.491
Between sides within times	12	0.509
Between treatment temperatures	4	4.812**
Differential effect of temperature \times time	12	0.597**
Sampling and analytical error within sides	48	0.035

** Indicates 1% level of significance.

this it is concluded that variations in the properties of the individual piece or side, or in their bacterial content or flora, determine, to a large extent, the concentration of nitrite present after exposure to heat treatments comparable to that of smoking. This conclusion is in general agreement with results reported previously (3, 6).

The differences between the nitrite content following a given period of treatment at different temperatures of treatment, can be determined more exactly, since the same sides were represented at all temperatures. It is evident from Table II that the differences between temperatures and the variance attributable to the interaction, temperature \times time, were both highly significant. This means that not only is the nitrite content a function of the temperature of treatment, but also that temperature has a differential effect on the nitrite content, depending upon the duration of treatment.

The direction of these significant changes in nitrite content under different conditions is evident from the results reported in Table I, but is shown more clearly in graphical form in Fig. 1. In this figure the nitrite content is plotted on a logarithmic scale against the temperature of the treatment. The four curves shown represent the different periods of treatment. Since the results obtained at 20° C. were considered separately in the statistical analyses, and since nothing is known of the changes that occur in the temperature range from 20° to 40° C., the points obtained at these two temperatures have been joined by a broken line, although the nitrite-temperature relation is doubtless curved in this range.

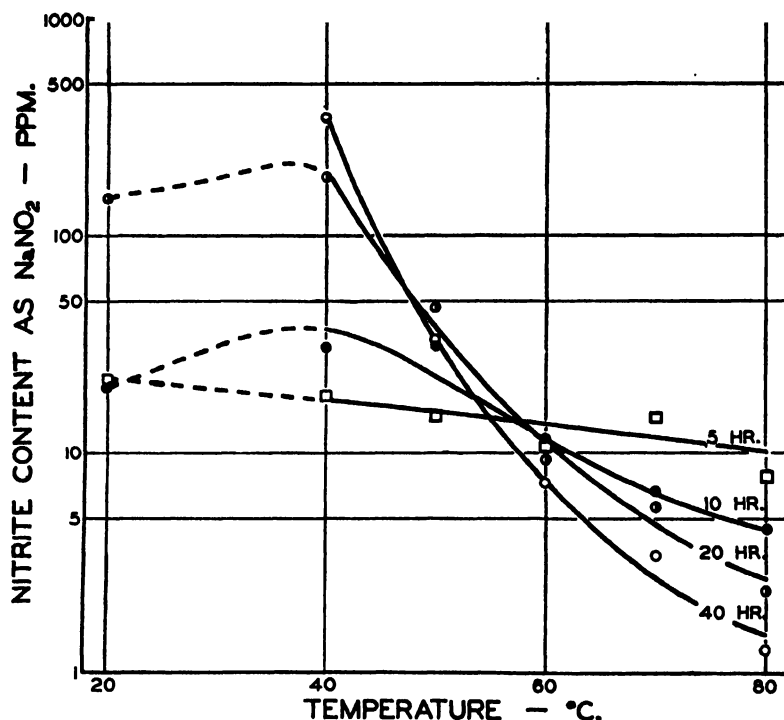


FIG. 1. *Effect of time and temperature of heating on the nitrite content of Wiltshire bacon.*

It would appear that the nitrite content, following a given period of treatment, is generally higher at 40° C. than at 20° C. Above 40° C. the nitrite content falls off more or less logarithmically as the temperature increases. Extending the period of treatment increases the nitrite content at 40° C., and decreases it at 80° C. The curves representing the various periods of heating, therefore, differ significantly in slope (Table II), and intersect one another in the region of 55° C., where the nitrite contents were approximately normal.

Discussion

Callow (1) has found that the nitrite content of small cuts of bacon may increase during storage for several weeks after curing. He found that the maximum nitrite concentration occurred at 5° C. and attributed the increase to bacterial action. The experiments reported in this paper were made over shorter periods at higher temperatures. In the present investigation the highest nitrite concentration occurred at 40° C., although the results at 20° C. suggest the possibility of a maximum nitrite production between 20 and 40° C. Since the optimum temperature for most bacteria lies between 20 and 40° C., it would seem reasonable to expect, if nitrite production is dependent on microbial activity, the maximum production of nitrite in this temperature range, rather than at cellar temperature.

In the main series of experiments (3), involving whole sides or large cuts, comparable increases in nitrite content were never observed, even in an individual side, during 10 days' storage at 1.1° C., smoking overnight at 40 to 45° C., and holding at room temperature for periods of 12 to 24 hr. In fact, smoking under these conditions tended to reduce rather than increase the nitrite content. These results suggest that time and temperature of treatment were not alone responsible for the increase in nitrite nitrogen observed in the present study.

It is possible that the proportionately greater surface exposed to the air in these small cuts may have been responsible for the observed behaviour. Certain direct evidence favouring this hypothesis was obtained from the observation that ground bacon stored at 4° C. showed a large increase in nitrite content within 36 hr., while the same bacon in the whole side showed no significant change. It appears, therefore, that the present results represent the effect of time and temperature under conditions that probably exaggerate their influence on nitrite production, as compared with those existing in commercial practice.

Although the results indicate that the nitrite content increased at temperatures below about 55° C., the agency responsible for these changes cannot be determined from these experiments. The accelerating effect of exposure to the atmosphere suggests that aerobic bacteria are responsible for nitrite production, but it is also possible that certain constituents or enzymes of the meat are capable of reducing the nitrate present to nitrite. Further work has been undertaken to determine the factors responsible for the observed increase in nitrite concentration.

Since protein coagulation occurs at temperatures in the vicinity of 55° C., bacterial and enzymic activity would be reduced to negligible proportions by exposure to higher temperatures. At temperatures above 55° C., the observed decrease in nitrite content may be attributed to chemical reactions causing a direct loss, oxidation, or combination of nitrite with the constituents of the meat.

Acknowledgments

The authors are indebted to the firms and individuals mentioned in the first paper of this series, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on the statistical treatment of the results.

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FURTHER EXPERIMENTS ON THE USE OF PHENOTHIAZINE AS AN ANTHELMINTIC FOR SHEEP¹

BY W. E. SWALES²

Abstract

The development and production of a disintegrating tablet as a practical means of employing phenothiazine as an anthelmintic for sheep are further described. Continued critical tests have shown high efficiencies against *Oesophagostomum*, *Haemonchus*, *Ostertagia*, *Monodontus*, *Nematodirus*, *Cooperia*, *Trichostrongylus*, and *Chabertia*. Tests against single infections with *Ostertagia* continued to show the complete efficiency of this form of treatment against this parasite. The periods of excretion of destroyed parasites with the faeces are noted. Single doses of phenothiazine did not anaematize animals under treatment.

The value of commercial phenothiazine as an anthelmintic for sheep was demonstrated during 1939. Harwood *et al.* (5) showed that, when mixed with food, the chemical was eaten by fasted sheep and resulted in the removal of a high percentage of hookworms (*Monodontus trigonocephalus*), nodular worms (*Oesophagostomum columbianum*) and probably stomach worms (*Haemonchus contortus* and *Ostertagia circumcincta*). Swales (8) reported tests showing high efficiency against the above mentioned species, and also demonstrating the efficient removal of *Trichostrongylus* spp., *Cooperia* spp., *Nematodirus* spp., and *Chabertia ovina*. During these tests a practical method was evolved for administering the drug to sheep; chemicals were ground and mixed with commercial phenothiazine and the mixture then compressed into hard tablets or slugs. Upon being placed in water or in the rumen of a sheep the tablet rapidly disintegrated and small particles of the insoluble phenothiazine were immediately dispersed. This method did not require prior fasting of the animals, required only about one-half minute to administer a therapeutic dose, and appeared to enhance the anthelmintic effect.

Roberts (6) and Gordon and Whitten (2, 3) reported tests of phenothiazine which demonstrated high efficiency against *H. contortus* and variable efficiency against *O. columbianum* and *Trichostrongylus* spp. Roberts administered relatively small doses of the chemical as a suspension in liquid paraffin, relying upon the prior stimulation of the reflex closure of the oesophageal groove by a solution of copper sulphate to direct the dose into the abomasum. In these experiments the efficiency against *O. columbianum*, with 4 gm. of phenothiazine in each dose, ranged from 8.8 to 94.7% in seven lambs. Roberts (7) also made extensive tests using a commercial preparation of phenothiazine ("Thiox"). He concluded that at a dose rate of 0.15 gm. per pound body

¹ Manuscript received May 24, 1940.

Contribution from the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, and the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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weight this drug gave fairly satisfactory results against *O. columbianum*, and at a dose rate of 0.4 gm. the results were very good in grown sheep but not equally good in lambs. He found the bulk of the larger dose to be an objectionable feature. During these tests Roberts noted a high efficiency against *H. contortus* but no effect against *Trichuris* spp.

Habermann and Harwood (4) later reported further tests using recrystallized phenothiazine. In doses from 15.6 to 20.0 gm., an average efficacy of 21.4% for *Monodontus* (*Bunostomum*), 90.1% for *Oesophagostomum*, 47.9% for *Chabertia*, 100% for *Haemonchus* and 20% for *Ostertagia* was demonstrated. In doses of 22.0 to 31.75 gm. the efficacy was 95.1% for *Monodontus*, 83.9% for *Oesophagostomum*, 93.8% for *Chabertia*, 80% for *Haemonchus* and 40% for *Ostertagia*. (No *Ostertagia* were recovered from faeces prior to necropsy, the estimation being made from prior examination of ova). Doses of 20 gm. were only slightly effective in removing *Cooperia* and partially effective in removing *Trichostrongylus*; however, a dose of 25 gm. removed 85.2% of 338 *Trichostrongylus* from one animal.

The work previously reported (8) was continued and extended in an effort to determine the practicability of the use of this anthelmintic in commercial sheep flocks.

Commercial Phenothiazine in the Form of Compressed Tablets

Following the preliminary description of the compressed tablet, attempts were made to improve this method of medication. However, tests have shown that only minor changes were necessary in the formula, which is now as follows:—

Commercial phenothiazine	— 80 parts
Starch	--- 8 parts
Sodium bicarbonate	— 5 parts
Tartaric acid	— 4 parts
Sodium choleate	— 2 parts
Phenolphthalein (U.S.P.)	--- 1 part

In preparing tablets for experimental use a satisfactory routine was developed. The ingredients are weighed into a one-gallon ball or pebble mill so that one load contains 800 gm. of the formula. Four pounds of pebbles and a heavy chain are used for grinding and mixing in the mill. The mill is turned for 15 min. at 70 r.p.m. The powder mixture is separated from the pebbles through a screen and is compressed into lumps by means of a plant-juice extracting press at 2,000 lb./sq. in. The lumps are granulated through a 10 mesh/inch screen. The granular material is compressed into the final tablet by means of a foot-operated press which produces a leverage on a piston of 11.5 : 1 by means of a heavy curved pedal lever. The material is measured into a die, which is a heavy walled steel cylinder, 3 in. long by $\frac{3}{4}$ in. inside diameter at the top increasing uniformly to a diameter of 51/64 in. at the bottom; the compression is obtained by a preliminary pressure on the lever

followed by a forceful kick. The tablet is then expressed by moving the base plate and thus allowing the piston to push the tablet through an aperture in the base plate and into a receptacle. The tablets made in this way are almost cylindrical (2.3% attenuation), have a hard, polished surface and are convex at each end. A single operator can make up to 200 tablets per hour. The above mentioned machine will produce tablets from 9 to 12.5 gm. in weight, depending on the measure used for the granulated material. A tablet weighing 12.5 gm. is approximately 1.5 in. in length.

The administration of the tablets presents no difficulties. A simple metal speculum, with bars from $2\frac{1}{4}$ to $2\frac{1}{2}$ in. apart, is used for adult animals; the tablets are placed, one at a time, over the back of the animal's tongue, the speculum being immediately withdrawn after each tablet is administered.

The administration of four tablets to an adult sheep usually occupies about half a minute if the animal is held correctly by an assistant.

Further Tests of Phenothiazine (Tablets)

Further tests were conducted in a similar manner to those previously reported (8). None of the animals were fasted prior to treatment and they had access to food and water immediately afterwards. About four hours after administration of the dose a specially constructed harness was fitted on the animal and a canvas bag attached over the hind-quarters so that all faeces were collected.

For three days previous to treatment and for four days afterwards, haemoglobin estimations (Sahli) and total erythrocyte counts were made twice daily on Animals 1 to 5 inclusive. This was done in order to check the possibility that single large doses of phenothiazine could produce a transitory anaemia, as reported by Thomas *et al.* in rabbits (9) and by DeEds *et al.* in man (1). In none of these animals was there any indication of a decrease in haemoglobin or in the number of erythrocytes. None of the animals showed any symptom of toxic effects of the dose, and no lesions attributable to the treatment were discovered at autopsy.

The results of the tests on nine animals are presented in Table I, and the post-treatment periods in which the removed parasites were found in the faeces are shown in Table II.

It is of interest to note that of the 85 residual *Monodontus*, only 26 were females, all but two of this sex being found in Animal No. 9. Most of the specimens of *Monodontus* removed during the first 24 hr. were females, the reverse being true for the subsequent periods. This supports the similar observation made during the previous tests and may be of some significance in the determination of the mode of anthelmintic action of phenothiazine.

Tests against single infections of *Ostertagia circumcincta* have been made. Four lambs, previously used for other work, and which were free of other stronglyloid nematode parasites, enabled tests to be made without the necessity of sacrificing the subject after each treatment. The infections in Animals B,

TABLE I
ANTHELMINTIC EFFECT OF PHENOTHIAZINE ON INFECTED SHEEP

Animal	Parasites removed by treatment and recovered from faeces										Parasites recovered at necropsy															
	Weight (lb.)	Strongylid ova (per gram)	Dose (gm., tablet formula)	Date	Haemonchus	Ostertagia	Monodonitius	Nematoditus	Cooperia	Trichostrongylus	Chabertia	Oesophagostomum	Date of necropsy	Haemonchus	Ostertagia	Moniezia	Monodonitius	Nematoditus	Cooperia	Trichostrongylus	Strongyloides	Trichinwis	Chabertia	Oesophagostomum	Capillaria	
1 Aged F.	125	1,500	62	11/9/39	0	0	46	0	0	0	0	9	22/9/39	0	0	0	0	0	0	0	0	0	0	0	0	10
2 Aged F.	114	-	50	25/9/39	0	0	12	0	0	0	0	2	3/10/39	0	0	0	0	0	0	0	0	0	0	0	0	
3 Lamb M.	50	4,500	25	5/9/39	110	12	4	950	20	1,045	0	14	12/10/39	0	0	7	4	122	0	311	30	0	0	1	0	
4 Lamb M.	54	1,800	27	15/10/39	31	130	71	27	356	664	10	15	19/10/39	0	0	3	2	103	0	107	60	3	0	0	0	
5 Lamb F.	49	1,330	25	19/10/39	11	22	105	12	870	450	9	11	25/10/39	0	0	1	30	0	1,080	80	1	0	1	0	0	
6 Aged F.	96	-	48	19/11/39	0	0	23	1	40	70	0	1	23/11/39	0	0	1	0	0	230	0	20	0	0	1	0	
7 Lamb M.	64	1,200	32	18/12/39	0	124	260	4	0	110	16	25	12/1/40	0	0	0	1	29	0	110	10	0	1	2	0	
8 Lamb F.	66	200	33	8/1/40	7	12	25	163	0	143	6	0	12/1/40	0	0	2	8	200	0	67	0	23	0	0	0	
9 Lamb M.	62	1,200	31	29/1/40	0	10	167	0	21	650	10	28	7/2/40	0	0	0	40	0	0	70	0	0	2	0	0	
Total worms					159	310	713	1,157	1,307	7,332	51	105		0	0	14	85	454	230	1,745	200	27	3	4		
Estimated average percentage efficiency of treatment														100	100	0	89	72	85	81	0	0	94	96		

TABLE II
THE POST-TREATMENT RECOVERY OF PARASITES FROM THE FAECES

	Percentages of total for each species			
	8 - 24 hr.	24 - 36 hr.	36 - 48 hr.	48 - 72 hr.
<i>Haemonchus</i>	97.5	1.9	0.6	0
<i>Ostertagia</i>	66.1	33.9	0	0
<i>Monodontus</i>	64.1	16.3	12.5	7.2
<i>Nematodirus</i>	60.8	5.8	33.1	0.3
<i>Cooperia</i>	96.9	3.1	0	0
<i>Trichostrongylus</i>	53.2	38.1	8.1	0.6
<i>Chabertia</i>	43.1	27.5	29.4	0
<i>Oesophagostomum</i>	72.4	16.2	11.4	0

C, and D were induced by doses of 5,000, 50,000, and 50,000 infective larvae of *O. circumcincta* respectively, the origin of the larvae being faeces from Animal A which acquired the infection on a test plot. Before artificial infection the cultures were carefully examined for the presence of larvae of other species, with negative results. The results of the treatments are shown in Table III.

TABLE III
EFFECT OF PHENOTHIAZINE ON LAMBS INFECTED WITH *Ostertagia circumcincta*

Animal	Weight, lb.	Dose (gm. tablet formula)	Date	Infection (ova/gm.)	Post-treatment tests*
A	90½	36	6/11/39	250-900	Tests negative 8/11/39 to 29/12/39
B	96	48	13/12/39	200-400	Tests negative 14/12/39 to 19/2/40
C	61	30	3/1/40	200	Tests negative 4/1/40 to 1/3/40†
D	100	50	3/1/40	200-600	Tests negative 4/1/40 to 4/3/40

* All post-treatment tests were concentration and sugar flotation methods using 15 gm. of faeces.

† Of 13 tests, two revealed the presence of 2 and 3 strongyloid ova respectively (25/1/40 and 1/2/40).

The post-treatment faeces from A and C were not examined for the presence of worms. Sixty-five specimens of *Ostertagia* were recovered from Animal B, 20 in the first 19 hours, 44 between the 19th and 24th hours, and one between the 24th and 27th hours. The faeces from Animal D contained 119 specimens, of which 69 were recovered in the first 19 hours and 50 between the 19th and 24th hours. It is probable that these worms represented only part of the total, as many may have become unrecognizable before being excreted with the faeces.

Discussion of Results

The commercial phenothiazine used in the experiments included in Table I was approximately 95% pure phenothiazine. Thus the doses used, based on the pure form, ranged from 19.0 to 46.5 gm. and averaged approximately

28.0 gm. of the pure chemical. The consistently good results indicate that the tablet form of the dose, which rapidly disintegrates into very small particles in the stomachs, enhances the anthelmintic efficiency of the phenothiazine. The definite laxative effect of the dose is apparent within a few hours, and particles of phenothiazine can be detected in the faeces between the eighth and twelfth hours after dosing. Although, in these experiments, only worms actually recovered from the faeces are considered in comparison with those remaining in the intestinal tract in estimating results, the apparent efficacies against *Oesophagostomum*, *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Cooperia* are significantly greater than those obtained by Habermann and Harwood (4) with recrystallized phenothiazine given in feed or in capsules.

The complete removal of *Ostertagia* by the described method supports previous findings. It is probable that a considerably smaller dose would be adequate for this purpose. The application of the treatment of ostertagiasis will be of greater importance in other countries including Great Britain, as this parasite has not, so far, been found to be of economic importance in Canada.

Acknowledgments

This further work was made possible by a grant received from Le Comité des Recherches Agricoles de Québec. The machinery and chemicals were obtained through a special grant made by the National Research Council of Canada, and the author wishes to acknowledge with gratitude this essential assistance.

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THE FATE OF PHENOTHIAZINE IN THE SHEEP¹

BY H. B. COLLIER²

Abstract

Methods have been devised for the detection and estimation of phenothiazine and its derivatives in the faeces, urine, blood, and milk of sheep, following oral administration of the drug.

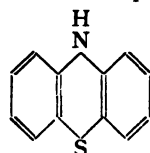
Only unchanged phenothiazine was detected in the faeces. In the urine was found a conjugate which was hydrolysed by strong acid in the cold. The conjugate was tentatively identified as potassium leuco phenothiazone sulphate; it was also present in the blood serum and the milk.

Catalase is inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazone.

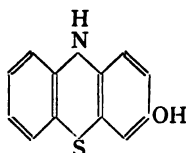
Introduction

The fate of orally administered phenothiazine in the rat, rabbit, and human has been discussed by DeEds *et al.* (2). They found that the substance was excreted in the urine as thionol, leuco thionol, and unidentified conjugates of phenothiazine and of leuco thionol.

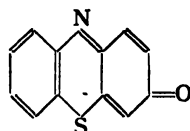
Phenothiazine is highly insoluble in water, but is soluble in alcohol and the fat solvents, crystallizing out in flat, pale yellow leaflets. It is changed to phenothiazone under mild oxidizing conditions and to thionol by stronger oxidizing agents. Both these derivatives are slightly soluble in water, giving an intense magenta colour, and both may be reduced to colourless leuco compounds. Eddy and DeEds (3) have described a quantitative method for phenothiazine, based on oxidation by bromine, and colorimetric estimation of the red colour produced.



PHENOTHIAZINE
 $C_{12}H_9NS$



LEUCO PHENOTHIAZONE
 $C_{12}H_9NSO$



PHENOTHIAZONE
 $C_{12}H_7NSO$

The present paper describes the examination of the faeces, urine, peripheral blood, and milk of sheep for phenothiazine and its derivatives, subsequent to administration of phenothiazine in tablet form, as described by Swales (7, 8). It seems highly desirable to identify the derivatives formed from phenothiazine in each species examined, in the hope that the findings may throw light on the pharmacology of the drug.

Following the identification of the chief excretory products in the sheep, quantitative methods have been devised and used to measure the rate of absorption and excretion. These methods are believed to be adequate for this purpose, although further work will doubtless result in refinements of the technique.

¹ Manuscript received May 24, 1940.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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Qualitative Examination

Faeces

Faeces from treated animals were extracted with hot water, but the filtered extract failed to show any colour, even after treatment with hydrogen peroxide or strong hydrochloric acid. This indicated that the water soluble oxidation products of phenothiazine, or their conjugates, such as may be detected in the urine by this procedure, were absent from the faeces.

Extraction with hot alcohol, followed by treatment with bromine water, yielded an intense red colour, suggesting the presence of unchanged phenothiazine. (Normal faeces gave no colour when treated similarly.) In fact, particles of phenothiazine were visible upon microscopic examination of the faeces.

Urine

The examination of the urine yielded results essentially similar to those described by DeEds *et al.* The urine when voided was of normal colour, but quickly turned red on exposure to the air. (If the coloured urine was allowed to stand in a flask, all but the surface layer slowly bleached to the normal colour, indicating reduction of the red compound.) The red pigment was extractable by chloroform and was assumed to be either phenothiazone or thionol.

Acidification of the urine with strong hydrochloric acid resulted in a very intense purple colour and the formation of a precipitate; this was apparently due to hydrolysis of a conjugate. The precipitate was soluble in alcohol and was oxidized by bromine to a red compound; it was therefore believed to be phenothiazine, as found by DeEds *et al.* However, the analysis and properties of the precipitate indicated, as described below, that it was not phenothiazine but a derivative.

Cooling of the urine caused the formation of a bulky, light coloured precipitate. This was found to be very soluble in hot water, and crystallized as sheaves of colourless needles. The crystals were soluble to the extent of about 1% in water at 20° C., and insoluble in alcohol and fat solvents. Treatment of the aqueous solution with strong hydrochloric acid and hydrogen peroxide gave a precipitate and an intense purple colour. The compound was therefore believed to be a conjugate; it was hydrolysed by strong acids, but not by weak acids or by alkalis.

A quantity of the conjugate was isolated from urine and recrystallized four times from hot water. The crystals did not melt sharply but darkened at about 220° C. A sample was submitted for analysis to Prof. Leslie Young of the Department of Biochemistry, University of Toronto. He reported as follows:

C = 43.12, 43.27%. H = 2.71, 2.74%. Ash = 23.85%. Potassium and sulphate were detected. Potassium leuco phenothiazone sulphate is a possible structure for the conjugate.

The author obtained the following analytical data:

N = 4.10, 4.14%. K = 9.4, 9.6%. Na = 0.90, 0.96%. SO₃ = 20.81, 20.97%.

The unhydrolysed conjugate gave a strong reduction with the Folin-Ciocalteu phenol reagent; the intensity of the reaction was not increased by hydrolysis.

Examination of the precipitate obtained on hydrolysis of the conjugate showed that it was appreciably soluble in hot water and in strong acids or alkalis. Thus, if a very dilute solution of conjugate was acidified, no precipitate formed. Phenothiazine itself is highly insoluble under these conditions. An alcoholic solution of the substance when poured into a small volume of water gave a greyish precipitate which showed an appreciable solubility, thus differentiating it from phenothiazine.

Treatment of the substance with sodium hydroxide and hydrogen peroxide resulted in the immediate appearance of an intense red colour; phenothiazine is only slowly oxidized under these conditions. This suggested that the precipitate from the hydrolysis of the conjugate is the leuco form of either phenothiazone or thionol. If a fairly strong solution of the substance in sodium hydroxide was treated with hydrogen peroxide, the red oxidation product precipitated out. This indicated the possibility of phenothiazone, rather than thionol, as the former is stated by Bernthsen (1) to be insoluble in alkali, the latter soluble.

A sample of this substance, recrystallized from dilute alcohol, was analysed by Professor Young, who reported the values for carbon and hydrogen given below. Total nitrogen was determined by the author.

Precipitate, found: C = 64.0%; H = 4.46%; N = 6.71%.

Leuco phenothiazone, calculated: C = 66.9%; H = 4.22%; N = 6.51%.

Phenothiazine, calculated: C = 72.3%; H = 4.55%; N = 7.03%.

These analyses, together with the properties described, indicate that the compound is probably impure leuco phenothiazone, and the conjugate the potassium salt of its ethereal sulphate. The analysis of the conjugate is summarized below, the calculated values being based on the formula C₁₂H₈NSO₃K = 333.3.

C,	found = 43.2%;	calculated = 43.2%
H,	found = 2.72%;	calculated = 2.42%
N,	found = 4.12%;	calculated = 4.20%
Ash,	found = 23.85%;	calculated = 26.13%
K,	found = 9.5%;	calculated = 11.73%
Na,	found = 0.93%;	calculated = —
SO ₃ ,	found = 20.9%;	calculated = 24.0%
Leuco phenothiazone		
(colorimetric),	found = 64.3%;	calculated = 64.6%

Blood

Blood serum showed only a trace of red colour on exposure to the atmosphere, but treatment with strong acid (sufficient to redissolve the protein) at room

temperature gave a deep mauve colour. This colour was slightly intensified by hydrogen peroxide but not by heating. The pigment was not extractable by chloroform, probably owing to combination with the protein. It was concluded that a conjugate is present in blood serum, presumably the same as that excreted in the urine.

Addition of *p*-toluenesulphonic acid to the serum precipitated the protein and hydrolysed the conjugate. It was demonstrated by the addition of this reagent to washed and haemolysed erythrocytes that they contained no conjugate.

Milk

The milk of lactating ewes treated with phenothiazine turned a definite pink on exposure to the air. Acidification with strong hydrochloric or *p*-toluenesulphonic acid resulted in a strong mauve colour, indicating the presence of the conjugate, as in blood and urine. Being water soluble, it was absent from the fat globules.

Quantitative Examination

An outline of the quantitative methods applied to the faeces, urine, blood, and milk of treated sheep is presented here. Results obtained by use of these methods are given in the following paper (9), where it is demonstrated that about one-half of the phenothiazine remains unabsorbed, and most of the remainder is recovered in the urine.

The colorimetric analyses were performed with the Klett-Summerson photo-electric colorimeter and the green filter No. 54. Since the colour intensities in the acid solutions did not strictly follow Beer's law, unknowns and standards of similar concentration were compared simultaneously. Alternatively, a calibration curve could be used. The leuco phenothiazine used as standard was prepared by recrystallization from alcohol of the precipitate obtained on hydrolysis of the urinary conjugate.

Faeces

The faeces were collected, dried at 110° C., ground and mixed thoroughly. The unchanged phenothiazine was determined by the following modification of the method used by Eddy and DeEds (3) for phenothiazine in spray-residues on fruit. Samples weighing 1 gm. were extracted by boiling with successive portions of 10, 10, and 5 ml. of 95% ethanol, the extracts being filtered, cooled and made up to 25 ml. Aliquots containing about 0.1 mg. of phenothiazine (as determined by preliminary tests) were transferred to a colorimeter tube graduated at 5 ml., and diluted to 2 ml. with 95% ethanol. About five drops of saturated bromine water was added and the excess bromine removed by boiling in the water bath. The solution was cooled, made up to the 5 ml. mark with 95% ethanol, and read in the colorimeter against a standard of 0.1 mg. of recrystallized phenothiazine in alcohol, similarly treated.

The blanks to be deducted from the observed readings of the unknowns were prepared from normal faeces similarly treated, and diluted to an equal extent. The alcoholic extracts of the faeces are a dark green colour, which disappears on treatment with bromine. For this reason, the extracts of faeces containing phenothiazine could not be used for blanks. By this method, phenothiazine added to normal faeces could be recovered quantitatively.

Blood Serum

Various protein precipitants were tested and found to be unsuitable. Marshall (6) has used *p*-toluenesulphonic acid for the determination of sulphanilamide and its conjugate, and it was found suitable for the estimation of the leuco phenothiazone conjugate when used in very high concentration. Addition of 3 ml. of 30% *p*-toluenesulphonic acid to 2 ml. of serum, followed by a drop of 6% hydrogen peroxide to insure complete oxidation, gave maximal colour; heating did not increase the colour.

However, equally satisfactory results were obtained by the use of excess hydrochloric acid without precipitation of the proteins. To 2 ml. of serum in the colorimeter tube was added 3 ml. of concentrated hydrochloric acid and one drop of 6% hydrogen peroxide. The colour was compared with a standard of leuco phenothiazone similarly treated. (0.002% leuco phenothiazone gave a reading of 345.)

Serum diluted to an equal degree with water provided a suitable blank, as determined by addition of conjugate to normal serum: quantitative recovery was thus obtained. Even badly haemolysed serum could be analysed by this means, as verified by suitable controls.

Urine

The urine was heated above body temperature to dissolve precipitated conjugate, filtered, and diluted to a suitable extent as indicated by preliminary tests. To two volumes of diluted urine were added three volumes of concentrated hydrochloric acid and a drop of 6% hydrogen peroxide. The colour intensity was read in the colorimeter against a leuco phenothiazone standard, as before. The blank was 7 *N* hydrochloric acid. This method gives total leuco phenothiazone. Free leuco phenothiazone may be determined by oxidation with hydrogen peroxide, and comparison with a standard aqueous phenothiazone.

Milk

Milk was centrifuged and the defatted liquid used for analysis. Addition of hydrochloric acid developed the typical red colour but the solution was too turbid for colorimetric reading. Finally it was found that addition of three volumes of 30% *p*-toluenesulphonic acid to two volumes of milk gave a clear filtrate which could be read, after addition of a drop of 6% hydrogen peroxide. The values thus obtained for the concentration of conjugate in the milk were compared with those obtained from blood samples taken simultaneously.

Discussion

As indicated in the following paper, about one-half of the phenothiazine administered to sheep was excreted unchanged in the faeces. A major portion of the balance was recovered in the urine, having been oxidized, absorbed, and conjugated.

The site and mode of absorption are at present unknown. Absence of oxidation products from the faeces suggests either that phenothiazine is absorbed as such, or that it is oxidized in the gastrointestinal tract and immediately absorbed. Since phenothiazine is highly insoluble, the latter alternative seems more probable.

Phenothiazone appears to result from mild oxidizing conditions; according to Bernthsén (1) it is formed by oxidation with atmospheric oxygen, especially in the presence of alkali. It is therefore surmised that a portion of the phenothiazine is oxidized in the gastrointestinal tract to phenothiazone or its leuco form; this is promptly absorbed and the major portion is conjugated to ethereal sulphate and excreted through the urinary tract.

DeEds *et al.* (2) believe that phenothiazone is too insoluble to act as an intermediate in phenothiazine excretion. However, its solubility is as great as that of thionol; the author estimated a solubility of about 0.01% at room temperature. Gersdorff and Claborn (4) used a 0.005% solution in their experiments, and Goldsworthy and Green (5) give the solubility as 0.004%. It is of interest to note that the latter authors believe phenothiazone to be the derivative possessed of fungicidal activity.

The findings with sheep differ from those of DeEds *et al.* with rats, rabbits, and human subjects, in that the phenothiazine seems to be excreted in the urine almost entirely in the form of the ethereal sulphate of leuco phenothiazone. No evidence was found for the presence of a conjugate of phenothiazine. Whether any of the free pigment in the urine is thionol was not tested; in any case it would be only a small fraction of the total excretory products.

In view of the confusion regarding the properties of these various derivatives of phenothiazine that occur in the animal body, it is felt that there is need for a more thorough investigation of their chemistry and pharmacology.

Acknowledgment

The author is indebted to Prof. Leslie Young, of the Department of Biochemistry, University of Toronto, for many valuable suggestions, and for the analyses performed in his laboratory.

NOTE. After the manuscript of this paper went to press it was found that liver catalase may be completely inhibited by leuco phenothiazone, leuco thionol, and thionol, but not by phenothiazine or phenothiazone. (It was also inhibited by sulphapyridine and by hexylresorcinol, but not by sulphanilamide.) This offers an explanation of the vermifugal and bactericidal

action of these compounds. The inhibition is probably due to the phenolic hydroxyl: leuco phenothiazone, leuco thionol, and thionol all reduced Folin's phenol reagent, but phenothiazone did not. The leuco compound isolated from the urinary conjugate and oxidized by aeration did not reduce the phenol reagent nor inhibit catalase, these observations affording further evidence that it is leuco phenothiazone.

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STUDIES ON EFFECTS AND EXCRETION OF PHENOTHIAZINE WHEN USED AS AN ANTHELMINTIC FOR SHEEP¹

By W. E. SWALES² AND H. B. COLLIER³

Abstract

Tests to determine the effects and excretion of phenothiazine and derivatives have been made on young sheep, barren ewes, pregnant ewes, and lactating ewes. Following the development of adequate technical methods, over 80% of the total dose was recovered from the faeces and urine in roughly equal amounts. Observations were made upon the occurrence of the maximum concentrations of total leuco phenothiazine in the blood, urine, and milk. No definite toxic effect has been noted. Further evidence of anthelmintic efficiency of phenothiazine is presented. The bacteriostatic effect of the derivatives in the milk is noted.

Eddy *et al.* (4), in a second publication on "Studies on phenothiazine," noted that a dye substance was excreted in the urine of white rats when these animals were dosed with phenothiazine. DeEds *et al.* (2, 3) and Thomas *et al.* (8), later made detailed studies of the nature and effects of the derivatives excreted through the urinary tract of rats, rabbits, and man. Harwood *et al.* (5) noted the red-stained urine from swine treated with phenothiazine, and listed this effect, as well as constipation, as host reactions to the drug. In the treatment of sheep with phenothiazine the excretion of the dye in the urine is an outstanding and somewhat objectionable characteristic.

During the studies on the anthelmintic effect of this chemical in sheep, observations have been made upon the effect and excretion of phenothiazine when administered in the form of disintegrating tablets as described by Swales (6, 7). Collier (1) undertook a study of phenothiazine and derivatives as they appeared in the blood, urine, faeces, and milk. The present study is a series of observations made upon animals after they had received therapeutic doses of commercial phenothiazine of approximately 95% purity, in the form of disintegrating tablets. Owing to the necessity of using this anthelmintic drug during the early spring months before the animals go to pasture, observations on pregnant and lactating ewes were of importance.

Technique

The collection of faeces was made by fitting a leather harness on the animal in such a way that one band encircled the neck, one the thorax and another the posterior abdomen; these bands were connected by a strap along the middle of the back and one along each side. Snaps were fitted on the posterior band and on the two side straps in such a way that a canvas bag, made to

¹ Manuscript received May 23, 1940.

Contribution from the Division of Animal Pathology, Science Service, Dominion Department of Agriculture, and the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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cover the hindquarters, could be easily attached and detached. By the use of this apparatus all the faeces were collected at intervals during an experiment.

Urine was obtained by enclosing the animal in an elevated pen and by attaching a rubber bag over the preputial orifice; a rubber tube leading from the base of the bag, through the pen floor and into a glass container, facilitated frequent collection.

The animal's head was held in a stanchion at the front of the pen, thus making the collection of peripheral blood from ear veins a simple matter. In collecting a sample of from 10 to 15 cc. the severance of a marginal branch of the greater auricular vein was found most satisfactory; the same incision could be used for several collections by simple manipulation.

The qualitative and quantitative determinations of derivatives were made by methods described by one of us (Collier (1)).

Preliminary Observations

The first observations were qualitative. During critical testing of the anthelmintic effect of phenothiazine tablets, particles of commercial phenothiazine were repeatedly found on microscopical examination of faeces passed between the 8th and 12th hours after treatment; such particles became more numerous during the next 12 hr. At necropsy particles were also found present in the stomachs and intestines four days after treatment. The urine passed between the first hour and the fourth day after treatment rapidly turned red on exposure to air. Tests of the blood on the 8th, 12th, 17th, and 24th hours after treatment of two animals revealed the presence of derivatives of phenothiazine. Tests were then planned in order to determine the relative amounts of the original dose that were excreted at various intervals through the intestinal and urinary tracts.

EXPERIMENT I (Jan. 29 to Feb. 7, 1940)

Subject: Ram lamb, weighing 62 lb.

Treatment: 31 gm. tablet formula (=23.6 gm. pure phenothiazine).

No fasting before or after treatment.

Analyses:

Blood	Total leuco phenothiazone.
	Positive in 1½ hr. after treatment.
	Maximum in 7½ hr. after treatment.
	Negative in 72 hr. after treatment.
Urine	Total leuco phenothiazone.
	Positive in 1½ hr. after treatment.
	Maximum in 6 hr. after treatment.
	Negative in 5 days after treatment.

Faeces not analysed; blood and urine quantitative estimations not accurate owing to imperfect methods. The efficiency against *Ostertagia* and *Oesophagostomum* was 100%, the former being all discharged in the faeces in the first 24 hr. and the latter in 48 hr. At necropsy no sign of toxic effect could be detected by macroscopic examination; the kidneys were examined for evidence of concretions of conjugate in the tubules, with negative results.

EXPERIMENT II (Feb. 7 to 13, 1940)

Subject: Male, aged 2 years, weighing 123½ lb.

Treatment: 61.8 gm. tablet formula (=47.0 gm. pure phenothiazine). No fasting.

Analyses:

—	Post-treatment times, hr.	Total leuco phenothiazine, %
Blood serum	1	0.0016
	5	0.0053
	9	0.0074
	24	0.0057
	48	0.0030
	72	0.0025

Urine: Method not sufficiently accurate for records of quantitative determinations. Urine was positive after 1½ hr. and was still positive when the experiment was discontinued three days later.

Faeces: Method not accurate, but over 50% of the original dose of phenothiazine appeared to be present in the faeces passed during the first four days. The maximum was reached during the second day and the faeces collected on the fourth day were practically negative.

This animal was not sacrificed, so no accurate estimation of anthelmintic efficiency was made. A simple sugar flotation test of 15 gm. of faeces revealed only five strongyloid ova prior to treatment; a similar post-treatment test was negative.

EXPERIMENT III (Feb. 13 to 17, 1940)

Subject: Male, aged 10 months, weighing 115 lb.

Pure infection with *Ostertagia circumcincta*, 800 ova per gm. of faeces.

Treatment: 28.8 gm. tablet formula (=21.9 gm. pure phenothiazine, or 0.19 gm. per pound body weight). Half the dose used in Experiment II per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazine, %
	1½	0.0012
	5	0.0033
	24	0.0042
	48	0.0009
	72	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	5 - 24	523	0.63	3.29	173
	24 - 48	437	1.18	5.17	215
	48 - 72	435	0.25	1.10	46
	72 - 93	—	Trace	—	—
	93 -	—	Zero	—	—
Total			9.56		

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	gm.	mg./hr.
	0 - 1½	48	0.036	0.017	14
	1½ - 4	75	0.215	0.161	59
	4 - 5	70	0.472	0.330	330
	5 - 21	450	0.669	3.010	188
	21 - 29	375	0.653	2.450	306
	29 - 45	450	0.431	1.940	121
	45 - 69	675	0.138	0.930	39
	69 - 93	435	0.042	0.183	8
Total			9.02 (8.4 gm. phenothiazine)		

Recovery of Phenothiazine

Faeces	9.56 gm.	= 43.6%
Urine	8.40 gm.	= 38.4%
Total	17.96 gm.	82.0% recovery

Post-treatment Tests of Faeces for Residual Infection

Quantitative tests (Stoll technique) were negative on the first day after treatment. By methods of concentration and flotation in sugar solution, 47 ova of *Ostertagia* were recovered from 15 gm. of faeces on the first day, but thereafter for 15 days the faeces were free of ova. On the following 36 days (March 1 to April 5, 1940) from one to six ova were recovered from 15 gm. of faeces, but the concentration tests then became and remained negative. At the time of treatment the infection was of 63 days' duration, having been induced by the administration of 75,000 infective larvae of *O. circumcincta*.

EXPERIMENT IV (March 7 to 12, 1940)

- Subject:** Male, aged 10½ months, weighing 113 lb.
Pure infection with *O. circumcincta*, 400 ova per gm. faeces.
- Treatment:** 56.0 gm. tablet formula (=42.5 gm. pure phenothiazine, or 0.38 gm. per lb. body weight).
Double the dosage of Experiment III per lb. body weight. No fasting.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazine, %
	6	0.0072
	12	0.0048
	24	0.0034
	48	0.0024
	72	0.0004
	96	Zero

Faeces	Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		
			%	gm.	mg./hr.
	8 - 24	41	1.71	0.70	44
	24 - 48	460	3.75	17.30	720
	48 - 72	395	0.04	0.16	7
	72 - 96	375	0.54	2.02	84
	96 - 103	87	0.014	0.012	1.7
	103 -			Zero	0
Total			20.2 gm.		

Urine	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazine		
			%	gm.	mg./hr.
	0 - $\frac{1}{2}$	35	0.014	0.005	10
	$\frac{1}{2}$ - 4	185	0.493	0.913	261
	4 - 6 $\frac{1}{2}$	193	0.580	1.120	448
	6 $\frac{1}{2}$ - 12	320	0.498	1.595	290
	12 - 24	600	0.770	4.620	385
	24 - 48	815	0.610	4.960	207
	48 - 72	710	0.256	1.815	76
	72 - 96	1400	0.046	0.642	27
	96 - 102	610	0.015	0.092	15
	102 -	—	Zero	—	0
Total			15.8 (14.6 gm. phenothiazine)		

Recovery of Phenothiazine

Faeces	20.2 gm.	= 47.5%
Urine	14.6 gm.	= 34.3%
Total	34.8 gm.	= 81.8% recovery

Post-treatment Tests of Faeces

Qualitative tests were negative on the day following treatment, and remained so for the following four weeks.

Discussion of Experiments I to IV

The above mentioned experiments have shown that between one-third and one-half of the total dose is excreted through the urinary tract. The early appearance (one-half hour) of derivatives in the blood and urine indicates that very rapid oxidation, absorption, and conjugation take place in the upper gastro-intestinal tract. In Experiment IV it will be noted that the sheep was constipated at the time of dosing and that this condition persisted for the first 24 hr. This temporary constipation delayed the faecal output of phenothiazine, but had no apparent effect upon the blood and urine levels of total leuco phenothiazone. However, if the constipation had not been corrected by the phenolphthalein content of the formula it is possible that a larger proportion of the dose would have been absorbed and excreted through the urinary tract, and that less phenothiazine would have been available for anthelmintic action in the lower intestines. This hypothesis constitutes the rationale for continuing the use of relatively large doses of the drug accompanied by a laxative, if high efficiency is to be maintained.

The maximum concentration of derivatives occurred in the blood and urine after about six hours, presumably when the particles of phenothiazine had spread throughout the gastro-intestinal tract. The blood and urine curves were almost parallel, indicating that excretion closely follows absorption.

The blood levels almost certainly indicate the intensity of absorption. In Experiment III the dosage was reduced to half that used in Experiments II and IV, and the concentration in the blood was noted to be proportionately reduced. However, it is of interest to note that the same fraction of the total dose was absorbed in Experiments III and IV.

The maximum faecal excretion occurred later than the maximum in the urine. The urine probably remains positive as long as particles of phenothiazine are present in the gastro-intestinal tract but the small amounts of blood conjugate are not detectable in the later stages. However, the possibility of concretions of the conjugate forming in the urinary tract must not be overlooked, even although such an occurrence has not been noted during the experimental work on this drug.

The high concentration of leuco phenothiazone in the urine of sheep, leading to rapid oxidation to an intensely red dye substance on exposure to air, constitutes an objectionable feature in the practical use of the drug. In the case of sheep used for commercial wool production, steps must always be taken to avoid urine contamination of wool for four days after treatment; prior shearing or the use of an abundance of pervious bedding are suggested.

Experiments III and IV have produced further evidence to show the complete efficacy of this treatment against *Ostertagia*, even with a very much reduced dosage.

The Treatment of Breeding Animals and Excretion of Phenothiazine Derivatives in the Milk

Previous tests having shown that phenothiazine, administered in the described manner, did not result in any discernible toxic effect on young animals and barren females, the question arose as to possible effects upon pregnant ewes, lactating ewes, and lambs nursed by treated ewes.

EXPERIMENT V

A preliminary test was conducted in August 1939, on two ewes, one weighing 137 lb., and nursing eight-weeks-old twin lambs, the other weighing 126 lb. and nursing one lamb of the same age. The ewes received 51.4 gm. and 47.2 gm. respectively of the tablet formula ($=0.28$ gm. per lb. body weight of pure phenothiazine).

The anthelmintic effect was good, the egg counts dropping from 600 and 200 ova per gm. to a total recovery from 15 gm. of 2 and 3 ova respectively. No effect on the lambs could be detected.

EXPERIMENT VI

Further tests were made in March 1940, as presented in Table I.

TABLE I

HISTORIES OF PREGNANT OR LACTATING EWES TREATED WITH PHENOTHIAZINE

Ewe	Treatment (gm. tablet formula = 80% commercial phenothiazine)	Date	Parturition	Interval	Lambs
(1) Pregnant	50	15 March	27 March	12 days	1 normal
(2) Pregnant	50	15 March	21 March	6 days	2 normal
(3) Pregnant	56	18 March	3 April	16 days	2 normal
(4) Pregnant	60	19 March	20 March	1 day	2 still-born (severe dystokia)
(5) Lactating	50	1 April	27 March	—	2, 1 still-born 1 normal
(6) Lactating	55	11 April	21 March	—	3, 1 still-born 2 normal
(7) Lactating	55	11 April	23 March	—	2 normal
(8) Lactating	Control	—	26 March	—	2 normal

It is possible that the laxative effect of the treatment may have accelerated the oncome of parturition in the case of Ewe No. 4, and may have been a contributing factor in the loss of the two foetuses. Blood was obtained from one foetus and was found to be free of leuco phenothiazone.

No adverse effect upon the other ewes and lambs was noted, all the lambs being very thrifty.

Ewe No. 4 was then used as a subject for further studies on the presence of derivatives of phenothiazine in the milk. It was noted that the milk turned to a pink colour after being exposed to air and light for several hours, and that it did not decompose when left standing in a warm room for several days.

EXPERIMENT VII (March 26 to 30, 1940)

Subject: Lactating Ewe (No. 4).

Treatment: 50 gm. tablet formula (= 38 gm. pure phenothiazine).

Analyses:

Post-treatment times, hr.	Total leuco phenothiazone, %	
	Blood serum	Milk
4½	0.0056	0.0029
11	0.0054	0.0090
24	0.0025	0.0140
48	Trace	0.0045
55	—	0.0024
72	Neg.	0.0006
80	Neg.	Trace
96	Neg.	Neg.

At each collection all the available milk was removed from the udder.

EXPERIMENT VIII

Subject: As in Experiment VII.

Treatment: As in Experiment VII.

Analyses:

Blood serum	Post-treatment times, hr.	Total leuco phenothiazone, %		
	6½	0.0098		
	24	0.0044		
	48	0.0010		
	72	Zero		

Milk	Post-treatment periods, hr.	Vol., cc.	Total leuco phenothiazone		
			%	mg.	mg./hr.
	0 - 1	13	0	0	0
	1 - 2	21	0.0008	0.2	0.2
	2 - 3	19	0.0026	0.5	0.5
	3 - 6	57	0.0112	6.4	2.1
	6 - 8½	40	0.0140	5.6	2.2
	8½ - 24	315	0.0145	45.7	3.0
	24 - 48	540	0.0038	20.5	0.9
	48 - 56	200	0.0005	1.0	0.1
	56 - 72	420	0	0	0
Total			79.9 mg. (0.21% of dose)		

EXPERIMENT IX (April 1 to 3, 1940)

This experiment was conducted in order to find if milk withdrawn normally by a lamb would continue to be positive for leuco phenothiazone for a period comparable with those in Experiment VII. The test was qualitative, simple acidification by two parts of hydrochloric acid being used to produce a colour reaction.

Subject: Lactating Ewe (No. 5).

Treatment: 50 gm. tablet formula (38 gm. pure phenothiazine).

Post-treatment times, hr.	Test for leuco phenothiazone
2	Positive
18	Strongly positive
20	Strongly positive
24	Strongly positive
26	Strongly positive
42	Trace
45	Negative

This experiment appears to indicate that when the milk is completely withdrawn by a normal lamb the leuco phenothiazone content may more closely parallel that in the blood than is indicated in the two previous experiments.

Discussion of the Studies in Milk

The above mentioned experiments show that, although derivatives of phenothiazine are excreted in the milk of lactating ewes, the total amount of such derivatives is too small to be of any significance in so far as direct effect upon the lamb is concerned. The evident bacteriostatic effect of the derivatives in the milk may be of interest, and warrants further study.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

AUGUST, 1940

NUMBER 8

CANADIAN WILTSHIRE BACON

XII. EFFECT OF HEAT TREATMENT ON THE COLOUR AND COLOUR STABILITY OF BACON¹

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Abstract

The colour of quadruplicate pieces of factory-cured bacon heated at temperatures of 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr. was measured at the conclusion of heating, and also after subsequent exposure of the samples for 12, 20, and 96 hr. at 10° C. and 95% relative humidity. Interacting effects of time and temperature of heating on colour at the conclusion of treatment were demonstrable. At 40 and 50°, total intensity increased with the duration of heating; at 60 and 70°, there was no definite trend, and at 80° it diminished markedly as the period of heating was prolonged. The maximum average intensity resulted from heating at 70°.

The decrease in intensity after 12 hr. exposure was related to the duration, but not to the temperature, of previous heating. There was no significant change in intensity between 12 and 20 hr., but a further decrease was evident at the end of 96 hr. The decrease in green intensity was still related to the duration, rather than to the temperature, of heat treatment, but the effects of duration of heating on red and blue stability, noted at 12 and 20 hr., were now replaced by temperature effects.

Partial correlation coefficients indicate that increased nitrite content of the meat at the conclusion of heating tended to be associated with a lower intensity of colour. On the other hand, both nitrite content and loss in weight (chiefly moisture) on heating were correlated with increased colour stability on exposure.

In the preceding paper of this series (2), White, Cook, and Winkler describe the details of an experiment designed to investigate the effect of heating Wiltshire bacon, as in smoking, for various periods at different temperatures, on its nitrite content and colour. The experiment comprised four periods of heating, namely, 5, 10, 20, and 40 hr., and six temperatures, 20, 40, 50, 60, 70, and 80° C., or 24 treatments in all. Four out of 16 "backs" cut from individual factory-cured sides were allotted at random to each of the four periods of heating, and each back was subdivided into six portions which were allocated, also by a random process, to the six temperatures maintained for each period of time. The above-mentioned authors conclude that heating at 55° C. resulted in an approximately normal nitrite content. Temperatures

¹ Manuscript received March 21, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as Paper No. 49 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 932.

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below 55° resulted in increased amounts of nitrite being found on analysis, whereas higher temperatures diminished the concentration of this constituent. These effects of temperature were accentuated by prolonging the time of heating.

It is now desired to describe the results of colour measurements of the 96 individual samples after treatment, secured by means of the photoelectric comparator employed in other phases of this investigation, already described elsewhere (3). The effect of heat treatment on colour and colour stability will first be dealt with, after which the correlation between these colour measurements and the foregoing determinations of nitrite content will be discussed.

Effect of Heat Treatment on Colour

The photoelectric comparator was employed as before to determine the component intensities of red, green, and blue light, relative to the white standard, reflected or scattered from the surface of the cut samples (4), and the sum of these three relative values was again taken as the index of colour intensity or total brightness.

Intensity or Total Brightness

Table I shows the results of an analysis of the variance (1) of the colour intensity as defined above, at the conclusion of heating of the 80 individual samples treated at temperatures of 40 to 80° C., and Table II the treatment averages, as well as the means by periods, for all temperatures, and by temperatures, for all periods. As was explained by White, Cook, and Winkler (2) the effect of temperature, which is deduced from comparisons between subsamples of the same sides, is more accurately determined than that of period of heating, which is affected by the variability between sides. This is reflected in the magnitude of the mean square errors (*a*) and (*b*) in Table I.

The analysis of variance demonstrates a highly significant effect of temperature on subsequent intensity of colour, and reference to Table II will indicate that the mean intensity, averaged for all periods, increased with

TABLE I

ANALYSIS OF VARIANCE OF COLOUR INTENSITY OR TOTAL BRIGHTNESS
OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square
Period of heating (all temps.)	3	261.0
Error (<i>a</i>) (between sides)	12	303.8
Temperature (all periods)	4	3,273.0***
Interaction, temp. \times period	12	618.6***
Error (<i>b</i>) (within sides)	45	39.0

*Exceeds mean square error, 0.1% level of significance.

TABLE II

AVERAGE COLOUR INTENSITY OR TOTAL BRIGHTNESS OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
	20	40	50	60	70	80	
5	121	120	126	154	163	168	146
10	115	115	134	155	163	158	145
20	113	128	139	152	157	143	144
40	—	143	158	161	169	128	152
Mean	116	126	139	156	163	149	147

temperature from 40 to 70°, falling off again however at 80°. The means for the four periods of heating, when taken over all five temperatures, do not differ significantly, but there is a significant interaction of temperature and duration of heating. Thus at 40 and 50°, the samples exhibited an increasing intensity of colour as the duration of treatment was prolonged. At 60 and 70° there was no appreciable trend, and at 80° the trend was the reverse of that noted at 40 and 50°, the brightness diminishing markedly with length of heating at this temperature.

For the reasons previously stated (2), the observations made on the samples heated at 20° C. were not included in the foregoing analysis of variance. A separate examination of these data did not indicate any significant difference in the brightness of the samples held for 5, 10, and 20 hr. at this temperature.

Chroma or Colour Quality

Tables III, IVa, IVb, and IVc give the analyses of variance and average values of the component red, green, and blue intensities of the samples subjected to the various treatments, in the same form as that adopted in Tables I and II above.

TABLE III

ANALYSIS OF VARIANCE OF COMPONENT INTENSITIES OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		Red	Green	Blue
Period of heating (all temps.)	3	39.7	50.5	30.3
Error (a) (between sides)	12	29.9	33.3	28.9
Temperature (all periods)	4	558.3***	336.2***	404.1***
Interaction, temperature × period	12	94.5***	61.7***	15.5
Error (b) (within sides)	45	9.6	5.1	11.6

***Exceeds mean square error, 0.1% level of significance.

TABLE IV. A

AVERAGE INTENSITY OF RED COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION
OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
	20	40	50	60	70	80	
5	49.0	46.0	49.0	61.0	64.9	65.6	57.3
10	46.4	46.0	53.4	62.7	65.7	61.4	57.9
20	43.7	50.8	53.9	61.0	62.4	57.8	57.2
40	—	57.2	62.7	63.8	66.5	50.9	60.2
Mean	46.3	50.0	54.8	62.1	64.9	59.0	58.2

The analyses of variance of red and green, and the treatment averages shown in Tables IVA and IVB, both reproduce features already noted in the results for total brightness, namely an increase in the average intensity for all periods of treatment with temperature up to 70°, followed by a reduction at 80°, and a significant interaction of temperature and treatment, intensity of colour increasing with duration of heating at 40 and 50°, and decreasing at 80°. There might seem to be some difference in the behaviour of the blue component, for its interaction mean square in Table III does not attain

TABLE IV. B

AVERAGE INTENSITY OF GREEN COMPONENT OF COLOUR OF BACON SAMPLES AT
CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
	20	40	50	60	70	80	
5	37.9	37.3	39.3	46.1	50.0	52.6	45.1
10	35.4	35.2	41.2	47.5	50.4	48.7	44.6
20	35.4	39.4	42.0	46.7	48.9	44.7	44.4
40	—	44.2	49.4	51.1	54.2	40.0	47.8
Mean	36.2	39.0	43.0	47.9	50.9	46.5	45.4

significance. Reference to Table IVc will, however, indicate that there is in fact some reversal of the time effect at the high and low temperatures, but that the effects of treatment in general are less pronounced in this component, and that the differences between the intensities recorded after heating for 5 and 10 hr., and between those for 20 and 40 hr., in particular, are for the most part quite insignificant. These comparisons, which contribute eight of the total 12 degrees of freedom for interaction, dilute the variance computed from the data as a whole, and when the remaining four degrees of freedom, representing the average interaction, with temperature, of heating for 5 and 10 hr. on the one hand and for 20 and 40 hr. on the other were isolated, they

TABLE IV. C

AVERAGE INTENSITY OF BLUE COMPONENT OF COLOUR OF BACON SAMPLES AT CONCLUSION OF HEAT TREATMENT

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
	20	40	50	60	70	80	
5	33.8	36.4	37.4	47.0	47.9	49.6	43.6
10	33.3	33.8	39.7	44.9	46.6	47.4	42.5
20	34.3	37.4	42.8	44.3	46.1	35.5	41.2
40	—	42.2	45.8	46.0	48.5	37.0	43.9
Mean	33.8	37.4	41.4	45.5	47.3	42.4	42.8

yielded a mean square of 34.9, significantly in excess of the mean square error (*b*), 11.6. It is to be concluded, therefore, that the treatments imposed influenced all three colour components in a similar manner, but that the magnitude of the effects was greatest in the red and least in the blue region.

The samples maintained at 20° C., which were again considered separately, failed to exhibit any statistically significant differences attributable to the period of heating.

Effect of Heat Treatment on Colour Stability

In addition to the foregoing observations at the conclusion of heat treatment, further measurements of the intensity of the red, green, and blue components of colour were made after the treated samples of bacon had been exposed in a light-proof chamber for 12, 20, and 96 hr., at 10° C. and 95% relative humidity. These enabled the effects of heat treatment on colour stability, which will again be considered under the separate heads of total intensity and colour quality, to be followed in some detail.

Intensity or Total Brightness

Tables V and VI summarize the results of the observations of change in total intensity as defined above, the former giving the analyses of variance (40 to 80°), and the latter the treatment averages for the 12-, 20-, and 96-hr. periods of exposure.

It will be noted at once from Table V that whereas the change in intensity after both 12 and 20 hr. exposure varied significantly in relation to the duration, but not the temperature, of heating, after 96 hr. these effects of heating period had disappeared. Curiously enough, the samples heated for the shortest periods showed the greatest decreases in intensity of colour after 12 hr. exposure (Table VI). There was no significant change in intensity between 12 and 20 hr.; in fact, the mean decrease observed after 20 hr., 5.2 units, was slightly less than the corresponding figure of 6.3 after 12 hr., but the difference is within the limits of the experimental error. By 96 hr., however, the mean decrease (at 19.0 units) was appreciably greater and, as noted above,

TABLE V

ANALYSIS OF VARIANCE OF CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square		
		12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	245.6***	323.3*	182.0
Error (a) (between sides)	12	18.9	58.7	60.8
Temperature (all periods)	4	11.7	9.9	135.6*
Interaction, temperature \times period	12	44.0	40.3	43.2
Error (b) (within sides)	45	47.3	45.2	45.1

* Exceeds mean square error, 5% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

TABLE VI

AVERAGE CHANGE IN COLOUR INTENSITY OR TOTAL BRIGHTNESS ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
		20	40	50	60	70	80	
12	5	-10.6	-11.2	-14.4	-10.0	-9.4	-5.2	-10.0
12	10	-3.2	-2.4	-12.4	-9.8	-7.0	-7.6	-7.9
12	20	-5.9	-8.2	-3.6	-5.3	-3.6	-6.1	-5.4
12	40	—	-1.2	-0.8	+1.4	-3.0	-5.7	-1.9
12	Mean	-6.6	-5.8	-7.8	-5.9	-5.7	-6.2	-6.3
20	5	-7.0	-7.2	-11.1	-9.2	-8.6	-5.8	-8.4
20	10	-0.8	-2.4	-9.8	-10.6	-8.8	-7.6	-7.8
20	20	-2.3	-5.1	-1.4	-7.9	-3.8	-7.3	-5.1
20	40	—	-0.8	+1.3	+5.6	-0.8	-3.2	+0.4
20	Mean	-3.4	-3.9	-5.3	-5.5	-5.5	-6.0	-5.2
96	5	-15.2	-15.4	-20.7	-22.4	-22.7	-18.1	-19.9
96	10	-7.9	-7.6	-19.5	-21.8	-21.4	-22.2	-18.5
96	20	-9.8	-14.8	-12.2	-18.6	-16.4	-13.7	-15.1
96	40	—	-21.0	-23.2	-22.5	-27.3	-17.8	-22.4
96	Mean	-10.9	-14.7	-18.9	-21.4	-22.0	-17.9	-19.0

the earlier effects of duration of heating had disappeared, but differences related to temperature had developed. These were such that the magnitude of the decrease in intensity increased with temperature of heating from 40 to a maximum at 70°, falling off again at 80°. There is thus a parallelism between the effects of temperature on colour at the conclusion of heating,

and on colour stability, which is worthy of remark in view of the correlation between initial intensity and stability noted elsewhere (5). In contrast to the effects on initial colour, however, there was no demonstrable interacting influence of temperature and duration of heating on stability.

Chroma or Colour Quality

Table VII gives the results of analyses of variance of the change in the component red, green, and blue intensities after exposure of the samples for 12, 20, and 96 hr. The results for the red and blue components resemble those already noted in the foregoing analysis of the changes in total intensity in indicating significant differences in stability after 12 and 20 hr. related to the duration of heating, which were replaced by differences ascribable to temperature at the end of 96 hr. Tables VIII and X further indicate that these effects were similar in nature to the ones observed in total intensity. Both of the components mentioned showed a greater reduction at 12 and 20 hr. following the shorter periods of heating, and a maximum decrease in intensity after 96 hr. in the samples heated at 70°.

TABLE VII

ANALYSIS OF VARIANCE OF CHANGE IN COMPONENT INTENSITIES OF COLOUR ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Variance due to	Degrees of freedom	Mean square (red)			Mean square (green)			Mean square (blue)		
		12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure	12 hr. exposure	20 hr. exposure	96 hr. exposure
Period of heating (all temps.)	3	26.6*	39.6*	23.1	18.0**	17.3	28.0*	47.6***	64.9**	22.0
Error (a) (between sides)	12	5.6	9.9	9.0	2.2	5.6	6.8	3.4	9.4	7.4
Temperature (all periods)	4	4.1	8.7	33.5*	1.7	1.3	5.6	5.8	3.0	18.4*
Interaction, temperature X period	12	12.3	9.5	8.9	5.2	4.9	3.5	4.7	4.7	6.0
Error (b) (within sides)	45	7.0	4.9	8.3	6.7	6.6	6.1	3.7	6.5	6.0

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

*** Exceeds mean square error, 0.1% level of significance.

The changes in the green component, on the other hand (Table IX), followed a somewhat different course, perceptible effects of duration of heating persisting even after exposure of the samples for 96 hr., whereas temperature effects were confined to narrow limits, which did not exceed the experimental error. After 96 hr. exposure, however, the decrease in green intensity of the various samples was no longer inversely proportional to the duration of heating. There was still a progressive diminution in loss of intensity from

TABLE VIII

AVERAGE CHANGE IN RED INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
		20	40	50	60	70	80	
12	5	-5.2	-3.2	-5.8	-4.0	-4.2	-0.4	-3.5
12	10	-1.8	-1.0	-4.4	-5.4	-3.4	-1.8	-3.2
12	20	-0.0	-2.6	-0.2	-1.5	-0.4	-2.4	-1.4
12	40	—	-0.0	-0.4	-0.6	-2.2	-3.0	-1.3
12	Mean	-2.3	-1.7	-2.7	-2.8	-2.5	-1.9	-2.3
20	5	-4.1	-1.3	-4.5	-4.0	-4.7	-1.8	-3.3
20	10	-1.6	-2.1	-4.8	-5.5	-4.8	-3.4	-4.1
20	20	+0.6	-2.2	+0.3	-4.4	-2.2	-4.1	-2.5
20	40	—	-0.6	-0.3	+0.8	-1.7	-2.2	-0.8
20	Mean	-1.7	-1.6	-2.3	-3.2	-3.4	-2.9	-2.7
96	5	-7.7	-5.8	-7.9	-8.9	-9.9	-7.2	-7.9
96	10	-4.4	-4.2	-8.3	-10.5	-10.8	-10.9	-8.9
96	20	-3.4	-7.0	-5.0	-9.3	-7.4	-6.7	-7.1
96	40	—	-8.9	-9.2	-9.6	-11.9	-8.2	-9.5
96	Mean	-5.2	-6.5	-7.6	-9.6	-10.2	-8.2	-8.4

TABLE IX

AVERAGE CHANGE IN GREEN INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
		20	40	50	60	70	80	
12	5	-4.1	-3.9	-3.3	-1.6	-2.5	-2.1	-2.7
12	10	-0.6	-0.4	-3.6	-2.2	-1.9	-1.6	-1.9
12	20	-2.4	-3.2	+0.1	-1.7	-0.6	-1.8	-1.4
12	40	—	-0.5	-0.5	+0.7	-0.6	-1.2	-0.4
12	Mean	-2.4	-2.0	-1.8	-1.2	-1.4	-1.6	-1.6
20	5	-2.6	-2.8	-2.2	-0.5	-0.8	-1.0	-1.5
20	10	+0.6	+0.1	-2.2	-1.8	-1.9	-0.4	-1.2
20	20	-1.4	-1.5	+0.5	-0.8	+0.2	-1.2	-0.6
20	40	—	+0.3	+1.1	+2.2	+0.2	-0.7	+0.6
20	Mean	-1.1	-1.0	-0.7	-0.2	-0.6	-0.8	-0.6
96	5	-5.2	-5.0	-5.7	-4.8	-5.9	-5.0	-5.3
96	10	-2.2	-1.9	-5.0	-5.4	-4.9	-4.1	-4.3
96	20	-3.0	-4.3	-3.0	-4.3	-3.3	-3.0	-3.6
96	40	—	-5.8	-7.0	-6.7	-7.4	-4.5	-6.3
96	Mean	-3.5	-4.3	-5.2	-5.3	-5.4	-4.2	-4.9

TABLE X
AVERAGE CHANGE IN BLUE INTENSITY ON EXPOSURE OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Exposure, hr.	Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-80°
		20	40	50	60	70	80	
12	5	-1.3	-4.2	-5.2	-4.4	-2.7	-2.8	-3.9
12	10	-0.8	-1.0	-4.4	-2.3	-1.7	-4.3	-2.7
12	20	-3.5	-2.4	-3.5	-2.1	-2.6	-1.9	-2.5
12	40	—	-0.7	+0.2	+1.3	-0.3	-1.4	-0.2
12	Mean	-1.8	-2.1	-3.2	-1.9	-1.8	-2.6	-2.3
20	5	-0.2	-3.1	-4.5	-4.8	-3.0	-3.0	-3.7
20	10	+0.1	-0.4	-2.8	-3.4	-2.0	-3.8	-2.5
20	20	-1.5	-1.5	-2.2	-2.6	-1.8	-2.0	-2.0
20	40	—	-0.5	+0.5	+2.5	+0.7	-0.3	+0.6
20	Mean	-0.5	-1.4	-2.2	-2.1	-1.5	-2.3	-1.9
96	5	-2.2	-4.5	-7.1	-8.7	-6.9	-5.8	-6.6
96	10	-1.3	-1.6	-6.2	-5.9	-5.7	-7.1	-5.3
96	20	-3.4	-3.5	-4.2	-5.0	-5.6	-4.0	-4.4
96	40	—	-6.3	-7.2	-6.2	-8.0	-5.0	-6.6
96	Mean	-2.3	-4.0	-6.2	-6.5	-6.6	-5.5	-5.7

the samples heated for 5 hr. to those heated for 20 hr., but, of all samples, the ones heated for 40 hr. now showed the maximum decrease. It is possible that after still further exposure, the decrease in green intensity following the various heat treatments might eventually have conformed to that of the other two components, but a further experiment would be required to test this point.

Correlation of Colour and Colour Stability with Moisture Loss and Nitrite Content

White, Cook, and Winkler having shown (2) that the nitrite content of the various samples was significantly affected by the heat treatment to which they were subjected, it was of interest to determine the extent to which changes in colour were related to the effects of heating on nitrite and moisture contents. These authors point out that the observed loss of weight of the samples on heating was an imperfect criterion of drying, as at the higher temperatures there was also some loss of melted fat which was absorbed by the paper wrapping. In the absence of more reliable data, however, the total loss in weight was of necessity adopted as the only available index of dehydration.

Table XI shows the coefficients of simple correlation between the loss in weight and logarithmic nitrite content (2) on the one hand, and the intensity and subsequent stability of colour on the other. There was a significant positive correlation between loss in weight and the intensity of red and green at the conclusion of heating, and a negative association between nitrite content

TABLE XI

COEFFICIENTS OF SIMPLE CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	r , intensity at conclusion of heating	r , change after exposure		
		12 hr.	20 hr.	96 hr.
Loss in weight \times total intensity	+ .26*	+ .26*	+ .10	+ .09
Loss in weight \times red intensity	+ .28**	+ .06	- .04	- .19
Loss in weight \times green intensity	+ .29**	+ .16	+ .10	+ .06
Loss in weight \times blue intensity	+ .19	+ .19	+ .12	- .06
Log. nitrite content \times total intensity	- .34**	+ .07	+ .23*	+ .25*
Log. nitrite content \times red intensity	- .37**	+ .19	+ .27*	+ .27*
Log. nitrite content \times green intensity	- .34**	- .10	- .01	+ .04
Log. nitrite content \times blue intensity	- .29**	+ .12	+ .09	+ .20

*Exceeds 5% point ($r = \pm 0.20$).

**Exceeds 1% point ($r = \pm 0.27$).

and all three colour components, the latter indicating that samples above-average in nitrite content tended to have a lower intensity of colour. When the changes in colour on exposure were considered, the only significant correlations in evidence were those between loss in weight during heating and the change in total intensity after 12 hr. exposure, and between nitrite content and the change in red and in total intensity after 20 and 96 hr. All of these coefficients are positive, indicating that above-average loss in weight and nitrite content were associated with greater stability of colour on exposure.

In this connection, however, it is again necessary to take account of the fact that there was a significant negative correlation between the loss in weight and nitrite content of individual samples, and significant positive correlations between the individual red, green, and blue intensities at the conclusion of heating, as well as between the changes in each of these components in any particular sample on exposure. For this reason, the partial correlation coefficients shown in Table XII were computed.

The partial coefficients for total intensity at the conclusion of heating suggest that the apparent correlation of this quantity with loss in weight (Table XI) was a consequence of the negative association, noted above, between loss in weight and nitrite content, the latter alone significantly affecting total brightness. There does seem to have been a significant relation between loss in weight and the individual intensities of green and blue, but as the correlation was positive in one case and negative in the other, the resultant effect on total intensity was inappreciable. Nitrite content, on the other hand, may be judged to have had a demonstrable effect on total intensity, but apparently no selective action on the individual components of colour.

The partial coefficients also bring out relations between loss in weight and nitrite content, and the change in intensity of colour after 12 hr. exposure, not apparent from Table XI. In this case, there were significant effects not

TABLE XII

COEFFICIENTS OF PARTIAL CORRELATION (r) BETWEEN MOISTURE LOSS AND NITRITE CONTENT, AND COLOUR AND COLOUR STABILITY OF BACON SAMPLES SUBJECTED TO HEAT TREATMENT

Quantities correlated	Independent of	r , intensity at con- clusion of heating	r , change after exposure		
			12 hr.	20 hr.	96 hr.
Loss in wt. \times total intensity	Log. nitrite content	+ .04	+ .42**	+ .36**	+ .37**
Loss in wt. \times red intensity	Log. nitrite content, green and blue intensity	- .06	+ .30**	+ .12	- .13
Loss in wt. \times green intensity	Log. nitrite content, red and blue intensity	+ .22*	- .28**	- .11	+ .11
Loss in wt. \times blue intensity	Log. nitrite content, red and green intensity	- .22*	+ .39**	+ .20	+ .07
Log. nitrite content \times total intensity	Loss in weight	- .24*	+ .35**	+ .42**	+ .43**
Log. nitrite content \times red intensity	Loss in weight, green and blue intensity	- .16	+ .45**	+ .31**	+ .14
Log. nitrite content \times green intensity	Loss in weight, red and blue intensity	+ .11	- .45**	- .24*	- .13
Log. nitrite content \times blue intensity	Loss in weight, red and green intensity	- .05	+ .43**	+ .19	+ .17

*Exceeds 5% point ($r = \pm 0.21$).

**Exceeds 1% point ($r = \pm 0.27$).

only on the change in total intensity, but also on the changes in red, green, and blue, each independent of the other two. This is a feature of some interest, for which no explanation seems to be readily forthcoming. It is also to be noted that additional loss in weight and nitrite content were associated with increased stability of red and blue over the 12-hr. period, but with a decreased stability of green.

Separate effects of nitrite content on red and green stability were still detectable in the observations made after 20 hr. exposure, but by 96 hr. these independent effects were no longer distinguishable, although there was still a significant correlation between nitrite content and the change in total intensity. Loss in weight during heating was likewise correlated with the total decrease in intensity up to 96 hr., but in this instance independent effects on the individual components apparently did not persist beyond 12 hr. of exposure. Both loss in weight, which was uncorrelated, and nitrite content, which was negatively correlated with the total intensity of colour at the conclusion of heating, were associated with enhanced colour stability on subsequent exposure.

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CANADIAN WILTSHIRE BACON

XIII. TENDERNESS OF BACON AND EFFECT OF HEAT TREATMENT ON TENDERNESS¹BY C. A. WINKLER² AND J. W. HOPKINS³

Abstract

Measurements made on uncooked samples of two factory-cured sides from each of 22 Canadian packing plants indicated significant differences between plants in respect of tenderness. The maximum average cutting force required by the product of an individual plant was more than three times the minimum plant average. The individual results however were fairly uniformly distributed over the observed range of variation, neither the maximum nor the minimum representing an isolated extreme. Partial correlation coefficients indicated a significant influence of pH of both pump and cover pickle, in conjunction with number of "stitches" and days in cure respectively, on tenderness, but no appreciable relation of this property to salt content, moisture content, nitrate content or pH of the meat, or number of days from curing to receipt at the laboratory, was demonstrable.

A second series of observations on samples heated at 20, 40, 50, 60, 70, and 80° C. for 5, 10, 20, and 40 hr., as in smoking, demonstrated significant interacting effects of the temperature and duration of heat treatment on tenderness. Excepting one notably anomalous result, the general tendency was for toughness to increase with the prolongation of temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. On the average, the maximum toughness was observed after heating at 50° C.

Introduction

In the course of a study of factors affecting the quality of Canadian Wiltshire bacon (1), determinations of the tenderness of two series of samples were made by means of the apparatus designed for this purpose by one of the authors (6). The two series comprised (i) samples of two factory-cured sides from each of 22 Canadian packing plants, and (ii) samples, of the same origin as in (i), subjected to various heat treatments, as in smoking.

As indicated in a previously published description (6) the apparatus actually provides a measure of toughness rather than of tenderness, namely the work required to cut through a piece of meat of specified width and thickness. The operation of the apparatus was the same as that previously described (6), and the data presented as indices of the cutting force are again the areas under the curve traced by the recording pen of the instrument in each case, corrected for thickness of sample. As these are directly comparable, they may be regarded as "relative work", without conversion into standard energy units. It was however found that over the range encountered in this series,

¹ Manuscript received March 21, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Published as Paper No. 50 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 933.

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the cutting force required varied more nearly as the square than as the actual thickness of individual samples. A Least Squares solution, based on 105 observations, actually indicated an average increase of 1.82 in the logarithm of the work done per unit increase in log thickness. This relation was accordingly used, in preference to the proportional factor previously adopted (6), to correct the observations for deviations of the individual test slices from the standard thickness of 5.0 mm.

Tenderness of Commercial Sides

Determinations were made on uncooked specimens from each of the 44 sides in series (i). For this purpose a sample of the necessary size was taken from the ham muscle of each side on arrival at the laboratory, the usual precautions being observed to secure representative material free from obvious striations of connective tissue (6).

After correction for thickness, an analysis of the variance (4) of the 44 resulting determinations indicated that the differences between the averages of the two sides from individual plants were significantly greater than would be expected from the observed variability of sides from the same plant. Table I summarizes the results of these observations, from which it is to be noted that the maximum average cutting force required by the product of an individual plant was as much as three times the minimum plant average. The individual results were however fairly symmetrically distributed over the observed range of variation, neither the maximum nor the minimum being an isolated extreme.

TABLE I
SUMMARY OF MEASUREMENTS OF TOUGHNESS,
CORRECTED FOR THICKNESS, ON SAMPLES
FROM 44 COMMERCIAL SIDES

Toughness	Relative cutting force required
Maximum	
Individual side	4.2
Plant (average of 2 sides)	3.6
Minimum	
Individual side	0.9
Plant (average of 2 sides)	1.2
Mean	2.4

As the sides from the various plants were not all of the same age when received at the laboratory, it would be incorrect to assume that all of the variation in tenderness of product between plants was ascribable to differences in the curing practices employed. Nevertheless, in view of the relation between tenderness and pH previously observed in samples of raw pork adjusted to

various degrees of acidity and alkalinity by injections of lactic acid or ammonia solutions (6), the effect of pH of pickle (3) on the toughness of the present series of samples was investigated. Omitting four plants for which the analytical data were incomplete, statistically significant correlation coefficients of -0.58 between the cutting force required and the product of pH of pump pickle (mean of two samples) \times number of "stitches", and of $+0.48$ between cutting force and the product of pH of cover pickle \times number of days in cure were obtained from the data for the remaining 18. There was no appreciable correlation ($r = -0.09$) between the two acidity indices for the same plant, with the result that the partial correlation of each index with toughness, independent of the other, at -0.61 and $+0.52$ respectively, did not differ significantly from the simple value. It is to be noted that the average pH of the pump pickles, at 7.6, was on the alkaline side of neutrality, and that of the cover pickles, at 6.6, was on the acid side, but in view of the fact that the pH of the meat itself on receipt at the laboratory, although previously shown to be affected to some extent by the pH of the pickles (3), nevertheless varied only within narrow limits, and could not be demonstrably related to toughness on the number of observations available, the mode of action of the pickles in modifying this last property remains obscure.

Coefficients of correlation between toughness and salt, moisture and nitrate content (2), as well as number of days from curing to receipt at the laboratory, were also computed, but failed to indicate any significant relation.

Effect of Heat Treatment on Tenderness

The heat treatments represented in Series (ii) comprised six temperatures, namely, 20, 40, 50, 60, 70, and 80° C., and four periods of heating, 5, 10, 20, and 40 hr. The experimental procedure, which was designed primarily to study the effect of heat treatment on nitrite content and colour, and has been described in detail elsewhere (5), may be summarized as follows: Sixteen "backs" cut from individual sides were allotted at random, four to each of the four periods of heating, and a piece from each back was allocated, also by a random process, to the six temperatures maintained for each period. The individual pieces, having been trimmed free of the cut portions of the ribs and the layer of back fat, were wrapped first in lightly waxed glassine paper and then in kraft paper, and heated in ovens maintained at the specified temperatures. At the conclusion of heating, the surface layers of each piece were trimmed off prior to the selection of suitable samples for the determination of tenderness.

Table II shows the analysis of variance of the measurements on the individual pieces, and Table III summarizes the treatment averages (means of four pieces). Unfortunately, through an oversight, the pieces which should have been heated at 20° C. for 40 hr. were removed at the end of 20 hr.; and those heated at 80° C. for 40 hr. were dehydrated to such an extent that satisfactory measurements of toughness could not be made. The tests of significance in Table II are therefore deduced from the data for 40 to 70°, in

TABLE II
ANALYSIS OF VARIANCE OF TOUGHNESS (RELATIVE WORK DONE
IN CUTTING) OF BACON SAMPLES SUBJECTED TO VARIOUS
HEAT TREATMENTS (40 TO 70° C.)

Variance due to	Degrees of freedom	Mean square
Period of heating (average for all temps.)	3	0.77
Error (a)	12	1.10
Temperature of heating (average for all periods)	3	3.96*
Interaction, temp. \times period	9	3.30**
Error (b)	36	1.02

* Exceeds mean square error, 5% level of significance.

** Exceeds mean square error, 1% level of significance.

which region the factorial combination of treatments was complete. Table III however includes all of the available observations.

Owing to the fact that the pieces cut from the same back resembled each other more closely than did those from different backs, the comparisons afforded by the experiment are of two levels of precision, as indicated by mean square errors (a) and (b) in Table II. Over the temperature range 40 to 70°, the differences between the average toughness after the four periods of heating did not attain statistical significance on the number of observations available. This however was due to a balancing of effects, rather than to an absence of any influence of duration of heating, as, in addition to average temperature effects, a significant interaction of temperature with period of heating is demonstrated in the lower part of this table.

The maximum average toughness, over all four heating periods, was observed after heating at 50° C., but owing to the interaction noted above the maximum for the individual periods varied from 60° for the 5 and 10 hr. periods to 40°

TABLE III
AVERAGE TOUGHNESS (RELATIVE WORK DONE IN CUTTING) OF BACON SAMPLES SUBJECTED TO
VARIOUS HEAT TREATMENTS

Duration of heat treatment, hr.	Temperature, °C.						Mean, 40-70°
	20	40	50	60	70	80	
5	3.05	4.05	3.40	4.25	4.28	3.02	3.99
10	3.02	3.75	3.78	4.40	3.02	2.98	3.74
20	3.63	4.35	4.65	3.75	3.48	1.58	4.06
40	—	3.65	5.85	2.78	2.08	—	3.59
Mean	3.31	3.95	4.42	3.79	3.21	2.52	3.84

for 20 hr. and 50° for 40 hr. With the exception of the anomalous value observed after heating at 40° C. for 40 hr., the general tendency was for toughness to increase with duration of heating at temperatures of 20 to 50°, but to decrease with the duration of temperatures of 60 to 80°. It has to be remembered of course that these results were obtained from small pieces of meat, and that the temperature and time effects noted are therefore unlikely to be directly applicable to the commercial treatment of entire sides. Nevertheless, the bearing of these facts upon the operations of commercial smoking will be appreciated, as temperatures ranging from as low as 40° in the smoking of bacon, to 60° or more in the production of "pre-cooked" hams are employed in practice. The reasons, other than possibly the straightforward removal of moisture, for the increase in toughness with time of heating at temperatures up to 50° are at present unknown, but it is of interest to note that the higher temperatures to which "pre-cooked" products are subjected may be expected to have a definitely tenderizing effect.

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PARTIAL CONGENITAL OCCLUSION OF THE VAGINA IN THE SILVER FOX¹

BY BENJAMIN KROPP²

Abstract

A condition of partially imperforate vagina in the silver fox is described and briefly discussed.

The present report deals with a congenitally anomalous urogenital orifice encountered in the silver fox (*Vulpes fulva fulva*). Two different males had been placed successively with the mature vixen, and each male had failed to impregnate her, owing apparently to failure of the female to receive the male intromittent organ. The owner then sacrificed the animal for its pelt since it was worthless as a breeder, and the carcass, in a frozen state, was turned over to the writer for examination.

The opening into the urogenital canal was found to be covered transversely by a dome-shaped septum that completely covered the external aperture except for a small opening at the extreme left lateral margin (Fig. 1). The

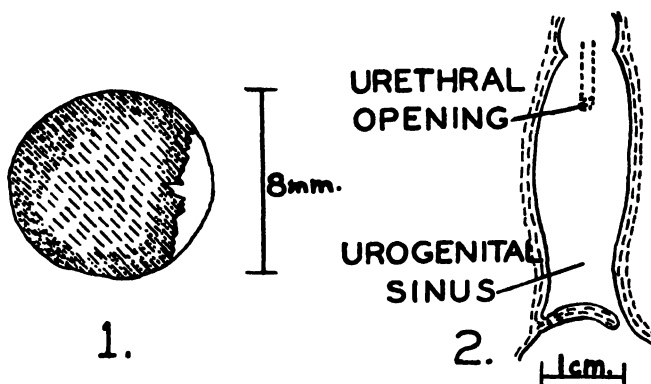


FIG. 1. Diagrammatic external view of membrane covering urogenital aperture. FIG. 2. Diagrammatic frontal section of urogenital sinus and membrane. See text for explanation.

septal edge bordering the marginal opening was uneven and serrated. The total diameter of the outer aperture in a lateral plane was 0.9 cm., and 0.8 cm. in an antero-posterior plane. The jagged free left edge of the septum

¹ Manuscript received April 22, 1940.

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reached to within 1.5 mm. of the left lateral margin. The membrane was tough and its external surface appeared cornified, although the membrane yielded readily to cutting with scissors. The concavity of the membrane may have been due in part to pressure of the penis during repeated attempts at copulation, but is to be attributed mainly to the effects of post-mortem shrinkage.

Unfortunately, the carcass was not fresh when received so that attempts to determine the nature of the occluding septum microscopically were not undertaken. However, the vagina and associated structures were dissected grossly and the septum found to be separable into two membranous sheets (Fig. 2), the inner sheet continuous with the vaginal lining, the outer sheet continuous with the skin surrounding the opening. Between the two sheets was a very thin layer of loose fibrous material, devoid of blood vessels, which sent small strands into the vaginal musculature. The ovaries, both horns of the uterus, and the fallopian tubes were grossly normal; no corpora lutea were detected in the ovaries. The urinary bladder opened into the urogenital sinus 28 mm. above the membrane, and the opening present in the latter was apparently sufficient to permit escape of urine. It is interesting to note that the presence of the septum could easily have been detected in the living animal if occlusion of the genital tract had been suspected as a possible cause of failure to mate. Removal of the structure with fine scissors could have been effected easily and sacrifice of the animal avoided.

Complete congenital occlusion of the vagina has been frequently encountered in laboratory colonies of adult rats (2) and mice (1). While in these and in other instances the congenital anomaly may vary in form, there is some reason to believe that in rats and mice the condition has an hereditary basis. The condition here described does not seem to be strictly comparable with that in rats and mice. The anomaly is regarded only as a developmental arrest in that the delamination of the vaginal plate failed to continue to completion.

Acknowledgment

The author is indebted for the specimen to Dr. N. V. Freeman.

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EXPERIMENTAL STUDIES ON *STRONGYLOIDES AGOUTII* IN THE GUINEA PIG¹

BY HENRY J. GRIFFITHS²

Abstract

The suitability and tolerance of the guinea pig to infection with *Strongyloides agoutii* presented an opportunity for the study of the bionomics of this species in an experimental host.

Serial transfer of this nematode through the guinea pig yielded a mixed type (free males and filariform larvae) of free-living development in faecal cultures which occasionally reverted to the indirect mode common to *S. agoutii*. A reversion to the indirect mode of development was produced when ova from faeces of guinea pigs infected with *S. agoutii* were cultured in sterile agouti faeces.

The termination of the prepatent period of *S. agoutii* in the guinea pig was shown to range from 7 to 10 days, and 71% of 58 animals were positive by faecal test by the eighth day. The patent period ranged from three to eight weeks.

The guinea pig was shown to develop an absolute acquired immunity to re-infection with *S. agoutii*. This resistance has been retained over a period of at least 6 to 13 months. An age resistance was not observed in animals one year old and over.

Introduction

Previous studies (10) on the morphology and biology of *Strongyloides agoutii*, of the golden-rumped agouti (*Dasyprocta agouti*), led to further investigation on the experimental infection of some common laboratory animals with this species.

For some time it has been known that the infection of certain abnormal hosts with *Strongyloides* may produce radical changes in the mode of free-living development. Brumpt (3) observed an alternation of the free-living cycle when he infected rabbits with *S. papillosus* of sheep. In sheep he observed a great predominance of females in the bisexual generation; in fact, free males were a comparative rarity. However, in rabbits the males were produced in large numbers, even exceeding the females, whereas the production of filariform larvae was strikingly reduced. Similar results were observed by the writer in cross-infection experiments on rabbits with the sheep strain of *Strongyloides*.

Sandground (13) recorded peculiar results through establishing *S. fülleborni* and *S. stercoralis* in abnormal hosts; the former, which normally showed only the pure indirect type of development, on inoculation into dogs and man produced predominantly the direct type. Observations of a similar nature were recorded by Faust and Kagy (6), who used human and non-human primate strains of *Strongyloides*. They transferred these strains to human volunteers, a macaque monkey, and a number of dogs, and their

¹ Manuscript received September 26, 1939.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

² Assistant.

results further demonstrated the instability of the free-living phase in hosts other than normal.

Accordingly, infection with *S. agoutii* was attempted in the guinea pig, which was selected for several reasons. Firstly, the guinea pig is a member of the same family as the agouti, and for that reason it was hoped that the presence of *S. agoutii* would be tolerated by this host. Secondly, the guinea pig does not appear to be readily infected with most helminth parasites and had been but little used as an experimental animal in helminthological research. As far as the writer is aware, there are only three recent references to infection of the guinea pig with *Strongyloides*: Krediet records an undetermined species from it; Brumpt (3) transferred *S. papillosus* from sheep to guinea pigs; and Sheldon and Otto (17) infected them with *S. ratti*, while testing the host specificity of this parasite.

Materials and Methods

The source of supply of *Strongyloides* material for this work was the golden-rumped agouti (*Dasyprocta agouti*), a rodent native to Trinidad, B.W.I., and northern South America. The housing, feeding, and handling of these rodents were described in a previous paper (10), as were the methods of making and examining the routine faecal cultures.

As a general rule, experimental animals were infected percutaneously with filariform larvae of *S. agoutii*, usually obtained from the lid of a Petri dish to which they migrated readily from the faecal mass. If not so readily available, the Baermann isolation technique was adopted with modifications. The majority of the larvae were isolated within an hour, provided the culture was not unduly thick and massive; by careful disposition of the culture, larvae and other free-living forms may be drawn off comparatively free of debris. This procedure would not be applicable to large masses, but is well adapted to small faecal cultures containing large numbers of larvae. *Strongyloides* larvae become quickly inactivated if stored in deep water. Therefore, it is preferable to draw them off frequently into Syracuse watch glasses and concentrate immediately prior to use for infection purposes.

A modification of this technique was also adopted as a confirmatory measure in some post-mortem examinations. On certain occasions it was desired to determine whether larvae of immature adults were present in the lungs, heart, and portions of small intestine.

As a general rule an anaesthetic was given when larvae were administered percutaneously. For complete anaesthesia, Nembutal was injected intraperitoneally at the rate of 1 cc. per 5 lb. of live weight; anaesthesia was complete in a few minutes, and lasted from 1 to 3 hr. A greater measure of success was observed by percutaneous than by oral administration of larvae. The guinea pigs were approximately eight weeks old unless required older for some specific experiment. A small area of about one square inch was shaved on the animal's abdomen, rinsed with water to remove soap, then with alcohol,

and allowed to dry. The infective larvae were applied to the spot by means of a pipette, and the area was supplied with larvae and moisture for 15 to 20 min. It was then permitted to dry and the guinea pig kept under observation until again active. On no occasion did this method fail to establish a positive infection when suitable host animals were employed.

The eggs of *S. agoutii* are in the late stages of segmentation or embryonated when evacuated in the faeces. The routine faecal examination was used for diagnostic purposes only; quantitative output of ova was not considered necessary for the interpretation of results. A sugar centrifugal-flotation technique was used, and about 1 cc. of faeces was taken for examination.

For collection of faeces the animals were kept individually in wooden cages with $\frac{1}{4}$ in. wire mesh bottoms. The faeces dropped through onto paper or into water, as required. At times it was desirable to collect the total output for several days. A four-sided funnel of sheet zinc was attached to the bottom of the cage by hooks; the seams of the zinc were soldered so that daily washing would remove dirt or urine sediment. A beaker was placed beneath to collect the pellets.

All infected animals were kept in metal cages with wire floors, and no contamination or re-infection of an individual was experienced throughout these studies. The guinea pigs were identified by their colour markings, as only small numbers were used at a time. Tattoo marks were used in the ears of rabbits and the ears and tails of rats and mice were cut for identification.

Prior to post-mortem examination, the animals were starved for at least 12 hr. The entire small intestine was removed by cutting at the pyloric end of the stomach and at the caecum. The duodenum was then separated and the remaining length of intestine divided into two approximately equal parts. Since the number of individuals obtained at autopsy was not significant in any of these experiments, a method of collecting and fixing the adults en masse was adopted. The portion of intestine was slit open with a sharp scissor blade and covered with normal saline in a small Erlenmeyer flask. After half an hour the flask was stoppered and shaken gently. This procedure was repeated two or three times and the intestine removed. Most of the worms were detached by this time and found among the floating mucus. The contents of the flask were poured slowly into a very fine tea strainer, which held back most of the nematodes, or into a strainer covered with bolting cloth, which retained all.

The worms were carefully washed off by a fine jet of water into a petri dish, in which they were preserved. The majority of the worms parasitized the upper half of the small intestine of the guinea pig, the greatest concentration being found in the duodenum and the first few inches of the jejunum. Adults were obtained frequently from the ileum but only on rare occasions from the caecum or colon.

All animals used (with the exception of the agouti) were born and raised at this Institute under conditions that precluded any possibility of previous infection with *Strongyloides* species.

The Infection of an Experimental Host with *S. agoutii*

The tolerance of the guinea pig to *S. agoutii* and its desirable qualities as a laboratory animal provided ample opportunity for investigation.

Experiments were outlined to yield data on the minimum time required for parasitic females to reach maturity, to note the type of free-living development that would occur after the passage of the strain through an experimental host, and to record the length of life of the infective larvae.

After primary infection with the agouti strain, infections were transferred serially from one guinea pig to the next. This procedure excluded the possible introduction of a new strain into the series and any change occurring in mode of development could thus be attributed to some change in type. Owing to the peculiar results recorded, it was considered desirable to repeat the serial infection of guinea pigs, and two analogous series were started.

By preliminary trial, the prepatent period of the parasitic females was found to be approximately eight days. Faeces samples were examined from the sixth day following infection. Faecal tests were continued daily until the individual showed a heavy output of ova, whereupon examinations were made weekly until the test was negative for two or three weeks, when examination was discontinued. The exact date that ova ceased to be passed was not recorded. On several occasions experimental animals appeared to become constipated at the time the female parasite commenced ova production. This unavoidable condition extended the prepatent period for one day or longer, in some instances.

As soon as large numbers of ova were detected, three samples per individual were collected on different days for culturing to observe the mode of development.

Because the ova are in the late stages of segmentation when expelled, it was not considered necessary to examine cultures for 20 to 24 hr. Examination of cultures was concluded as soon as the activity of the filariform larvae ceased. It will be noted that the total number of cultures recorded graphically does not always correspond exactly with the total number of cultures examined. These omissions were unavoidable on account of sickness and holidays which occurred during the investigation. Some animals employed in these studies died as a result of over-infection, some were killed to obtain specimens of the parasitic generation of *S. agoutii*, and others were held for future use in immunity studies.

Serial Infection of the Guinea Pig. Series I

In this series, serial transfer of the agouti strain of *Strongyloides* was made through 27 guinea pigs. The experiment extended from April 1936 to November 1937, and during that period 92 cultures were made and 1,314 observations recorded.

Filariform larvae used for infective purposes ranged from 4 to 17 days in age, though the majority were from 4 to 6 days old. All the animals were

young males except guinea pig No. 21, a three-months-old female. Cultures from No. 24 were not examined in detail, but filariform larvae were obtained for transfer purposes. No. 27 died suddenly, terminating this series.

Transfer of *S. agoutii* through the guinea pig resulted in a marked change in the mode of free-living development. In the agouti, this strain showed an indirect or heterogonic mode of development, whereas in the guinea pig a mixed type was observed in the first 19 guinea pigs. The term "mixed" is used to indicate that the development observed was not entirely direct, for in addition to the production of filariform larvae, free-living males made their appearance. Only occasionally were no males found.

This mode of development did not persist throughout the entire series, since cultures from No. 20 showed the presence of a few free females in addition to the filariform larvae and free males, a modified reversion to the indirect type of the agouti. Cultures from No. 21 continued to show the presence of free females in quite large numbers, though cultures from Nos. 22, 23, 25, and 26 produced only filariform larvae and free males.

This mixed type of development presented an excellent opportunity for recording the period of activity of free males, the first appearance of filariform larvae of direct development, and the longevity of these larvae, in faecal cultures. Such data are recorded in Figs. 1-4. Free males occurred in the majority of cultures on the second and third days, but only once on the first day; they did not appear after the fourth day (Fig. 1). In the greater number of cultures the males became inactive on the fourth and fifth days, though in one instance they were active on the seventh day. In only one instance were males observed in cultures for more than four days, the longevity in most cases being from two to three days. Filariform larvae occurred in the majority of cultures on the third day, though many were recorded on the second and fourth days; in a few instances they did not appear until the fifth day (Fig. 2). The duration of life of filariform larvae in cultures was easily recorded with this mixed type of development.

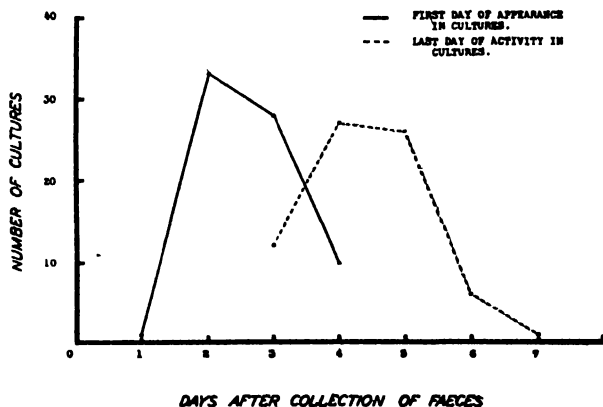


FIG. 1. Frequency of first day of appearance and last day of activity of free-living males of *S. agoutii* in faecal cultures from the guinea pig.

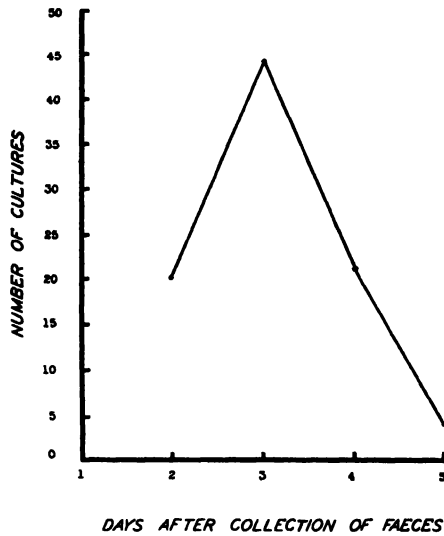


FIG. 2. Appearance of filariform larvae of *S. agoutii* in cultures after passage of faeces by guinea pigs.

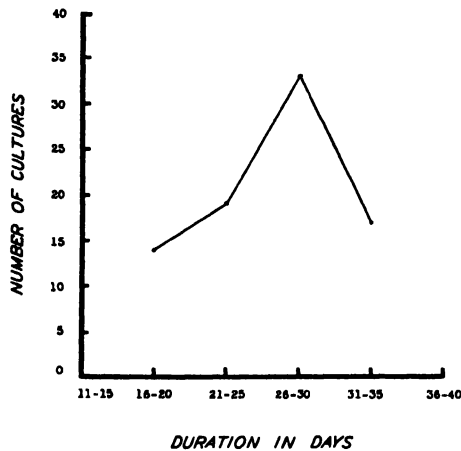


FIG. 3. Longevity of filariform larvae of *S. agoutii* in faecal cultures from the guinea pig.

Fig. 3 indicates that the greater proportion of cultures died between the 26th and 30th days, while no cultures became inactive prior to 16 days, or continued to show active larvae after 35 days. The appearance of filariform larvae on the third day in the greater number of cultures will permit the deduction that the maximum life of larvae is 32 days. No evidence of lack of viability of larvae was observed as the result of serial transfer.

Of the 27 guinea pigs in this series, 21 were killed and given a thorough post-mortem examination, one was killed accidentally and received no autopsy, and five were used for other investigations. Nine of the 21 were killed to obtain adult *Strongyloides*, while the other 12 were killed some months after infection in order to check the negative faecal tests that were being recorded.

None of these 12 animals revealed any stages of *Strongyloides* in the lungs, heart, liver, small or large intestine. It is therefore considered that the infection of these animals was eliminated rather than that the parasitic females were becoming non-fecund.

Serial Infection of the Guinea Pig. Series II and III

Owing to the peculiarities observed in the free-living phase of Series I, two further series were commenced to repeat the observations under conditions as nearly identical as possible. The data obtained relating to longevity of free forms and length of life of cultures displayed such similarity to the results shown graphically for Series I that they did not seem of sufficient importance to warrant repetition.

Series II extended from July 1937 to June 1938, and during this period *S. agoutii* was serially transferred through 15 guinea pigs, 92 faecal cultures were prepared, and 919 observations were recorded. Filariform larvae used for infection purposes ranged from 3 to 18 days in age; in most cases, four-day-old larvae were used. Guinea pigs Nos. 1 and 2 were the only females in this series.

The mode of development was very inconsistent. Cultures from Nos. 1 and 3 yielded a few free females in addition to the filariform larvae and free males. Cultures from No. 7, however, gave filariform larvae and free females almost exclusively and in large numbers, though one or two males were observed. All other cultures produced filariform larvae and free males only; in a few cases, no males were observed. Since no constancy in mode of development was apparent, the series was discontinued after the fifteenth individual was infected. Of the 15 guinea pigs, six were killed by over-infection or to obtain adult parasites, six were killed after some long period to check reliability of faecal examinations, and three were used for further studies.

The studies in Series III consisted of the serial transfer of *S. agoutii* through 12 guinea pigs, from January to July, 1938, and included 60 cultures and 804 observations. The larvae used for infection purposes ranged from 3 to 12 days old, but were usually four days old.

The type of development in Series III was also variable. A few free females were recorded from cultures from guinea pigs Nos. 3 and 10. The remaining cultures tended to show direct development, with free males recorded intermittently throughout. Because of the variable results, the series was concluded after infection of No. 12. Eight of the 12 animals were killed or died as a result of over-infection. Adults were obtained from five only, the other three being negative. The remaining four animals were used for further study.

The Free-living Development of *S. agoutii* from the Agouti and Guinea Pig

In a previous paper by the author (10) on *S. agoutii* of the agouti, the indirect mode of development of the free-living generation was found to prevail. In this study, it has been shown that a mixed type appears in cultures of

faeces from guinea pigs infected with *S. agoutii*. This mixed type is represented by filariform larvae and free-living males, though free females have appeared. Two types of free-living development have thus been shown for this strain of *Strongyloides* in different hosts.

An attempt was made to ascertain whether the factors influencing the type of development could be attributed to the host or to the environment of the culture media. A young male guinea pig was infected percutaneously with *S. agoutii* from the agouti. Upon appearance of ova in its faeces, three groups of cultures were made: (i) faeces; (ii) ova removed from faeces by crushing, sieving, washing, and sedimentation, were seeded on guinea pig faeces from uninfected young stock and (iii) on sterile agouti faeces (autoclaved at 14 lb. pressure for 15 min.). Sterility of this agouti faeces, and also of faeces from the uninfected guinea pigs, was verified by control cultures.

To obviate error in culture examination, these were examined daily under the binocular microscope, and a sample was examined by the Baermann technique. When cultures yielded only filariform larva or no larvae for five or six days, examinations were discontinued.

Of 10 cultures in group (i), all showed mixed development, as would be expected. In group (ii), 10 cultures all yielded filariform larvae and free males, with one free female in one culture. In group (iii), three out of four cultures showed the indirect mode of development, with one larva observed on the first day in one culture. A further 13 cultures in this group showed seven with indirect development, five with no development, and one with two larvae on the fourth day.

In the second and third groups, the population of the cultures was low in many cases. The uncontrolled process employed in seeding cultures with ova, which was not carried out quantitatively, may possibly explain this.

Discussion of Free-living Development of *Strongyloides*

In many instances when a species of *Strongyloides* is experimentally established in a new host of different taxonomic position from its usual or normal host, the life cycle is observed to be radically changed from the type manifested in the original host. Inadequate observations have on occasion led to the belief that a certain strain is exclusively direct or indirect, whereas actually few such strains appear to exist.

From the data obtained on the free-living phase of cultures from Series I, definite peculiarities in the mode of development were observed. By transfer of the agouti strain of *Strongyloides* through the guinea pig, a direct type of development might be expected. In these studies a modified direct mode was recorded, being represented by filariform larvae and free males. This mixed development was evidenced in cultures from the first 19 animals of the series, after which free females were recorded. Similar observations were recorded in Series II and III, though females occurred with considerable irregularity and inconsistency.

In an attempt to explain this phenomenon the current hypotheses will be reviewed as briefly as possible. Sandground (13) regards the parasitic female as syngonic, giving rise to ova which develop to males or females. If ova are fertilized in equal numbers, theoretically both sexes would be reproduced in equal numbers, but this seldom occurs. If only females or males are produced in the bi-sexual generation, he considers that the type of sperm that determines the mixing sex has either not been produced or has degenerated without functioning. Direct development might readily be attributed to parthenogenesis, but Sandground found the same syngonic condition occurred in parasites that were known to have been producing the direct type of development almost exclusively. However, the finding of sperm in his syngonic females does not completely exclude the occurrence of parthogenesis. Sandground further provides an explanation for direct development by consideration of the occurrence of an atypical distribution of the chromosomes at mitosis, which would constitute the determining mechanism for this mode. He suggests auto-infection as an explanation for direct development, on the hypothesis that in an infection originally showing both direct and indirect modes, the larvae of direct metamorphosis may, by auto-infection, reinforce the existing infection. Since larvae have been shown to develop mainly according to the parental history, it will follow that with the increasing age of an infection, the direct mode will finally predominate. Sandground (13) has observed in daily cultures of material from a rat, that within a short period, the sex-ratio may range from a bisexual generation almost entirely of females to another of males only. Following the revolutionary discovery of the parasitic male by Kries (12) and Faust (5), the latter proposed a possible explanation for alternative modes of development. He suggested that fertilized ova may give rise to an indirect mode of development, unfertilized to a direct mode. After the original supply of spermatozoa stored in the females becomes exhausted, the progeny of parasitic females (by parthenogenesis) in the intestine would be direct only, since the parasitic males are usually found in the bronchioles and consequently would not re-fertilize the females.

Following his investigation with *S. simiae*, Beach (1, 2) proposed the theory that under optimum conditions indirect development consistently occurred, whereas unfavourable conditions tended to modify the strain toward a direct type. Beach considers that the sex is established before or at the time of formation of the egg, but the development is modified by factors such as nutrition, viscosity, and toxicity. He further suggests that filariform larvae, appearing as a result of changing from an indirect to a mixed type are, in reality, potential sexual individuals. His work strongly indicates that directness and indirectness are conditioned by environment and not by genetic constitution. Graham (9), however, presents evidence indicating that the mode of larval development followed by *S. ratti* is determined prior to oviposition.

The occurrence of parthenogenesis in the first 19 experimental animals of Series I could readily explain direct development if no free males had been present. Chance hyperinfection of guinea pig No. 20 might have permitted fertilization of a parasitic female with subsequent production of indirect development. However, since the ova of *Strongyloides* are evacuated from the guinea pig in the late stages of segmentation (although usually embryonated in the agouti), it seems improbable that Faust's hyperinfection or Sandground's autoinfection would occur.

Parasitic males were not observed on any occasion, which supports the suggestion of Faust that unfertilized ova may produce a direct type of progeny. However, this fails to account for the females in the twentieth generation, and is inadequate to explain the free males throughout the series.

The conditions experienced by the *Strongyloides* strain in cultures of guinea pig faeces may have been sufficiently unfavourable to influence the strain to a direct mode of development. But as all guinea pigs received materially the same rations and all cultures were made under as nearly identical conditions as possible, this influence would be constant throughout the series.

Beach (2) considers that filariform larvae, produced when first-generation rhabditiform larvae are grown on artificial media, are potential free-living adults. He has demonstrated the alteration of a supposed indirect type of development of *S. simiae* to a mixed type when the worms were grown on artificial media. This mixed type showed large numbers of first-generation filariform larvae, a considerable number of males, and few or no females. On addition of small amounts of aqueous extracts of monkey faeces to the media, a more favourable growth was induced, and the mixed development tended to revert to the original mode, i.e., there was an increase in females and a decrease in the filariform larvae. These observations appear comparable with the data of the present study, and Beach's deductions are more readily applicable than any previously discussed. It seems logical to assume that indirect development is normal for *Strongyloides*, direct development occurring when unsuitable conditions are encountered. As a result of this adaptation, filariform larvae of the direct mode are considered as suppressed free females and males, which manifest their true forms only in the presence of certain definite requirements.

In these studies, the composition of guinea pig faeces apparently does not completely furnish the necessary medium for the appearance of free females and males with regularity, though males have appeared more consistently than females.

The results obtained from the culture of ova of *S. agoutii* from the guinea pig on sterile agouti and guinea pig faeces present strong evidence in support of the theory that directness and indirectness are conditioned by environment. These two cultures of ova displayed different modes of free-living development. The former reverted to the original indirect type of the agouti strain; the latter showed the development of free males and filariform larvae, as usually observed in cultures from a guinea pig infected with *S. agoutii*.

A number of cultures of agouti faeces seeded with ova from guinea pig faeces showed no development. This condition may probably be attributed to the uncontrolled method of distribution of ova on cultures or to some unknown factor preventing development of the ova.

The mechanical methods employed in obtaining these ova did not affect the mode of development of larvae. From the results obtained it is assumed that the factor controlling the mode of free-living development of *S. agoutii* is directly associated either physically or chemically with the media in which the eggs are cultured.

The Prepatent and Patent Periods of *Strongyloides agoutii* in the Guinea Pig

The prepatent and patent periods in members of the genus *Strongyloides* have been variously determined and have shown considerable diversity for different species and strains. Sandground (14) reported the prepatent period of *S. stercoralis* to be 5 to 10 days in dogs and 9 to 16 in cats. Faust *et al.* (7) recorded infections of dogs with human strains of *S. stercoralis* in which the prepatent period usually ranged from 11 to 18 days. Graham (8), using single larva infection of rats with *S. ratti*, showed the prepatent period to range from 5 to 11 days, most individuals being positive by culture on the sixth day, whereas Sheldon (15) recorded the prepatent period of *S. ratti* as ranging from 3 to 6 days.

In these studies, the three series of guinea pigs infested with the agouti strain of *Strongyloides*, together with a few other individuals employed in immunity investigations, gave considerable data on the period between infection and the appearance of ova in the faeces. All animals were tested on the sixth day after infection and daily thereafter. The frequency distribution of the first day of appearance of ova, after exposure to filariform larvae, is recorded in Fig. 4. In spite of the many factors that may impede the migration of larvae to the intestine or delay the discharge of eggs, it is of interest to note the narrow range to which the first appearance of ova is confined. Of the 58 animals, 24% were positive by faecal test on the seventh day, 47% on the eighth, 22% on the ninth, and 7% on the tenth day; that is, 71% were positive by the eighth day, the modal point for the series.

These observations show the commencement of the patent period to be confined to comparatively narrow limits; in fact, these limits are considerably narrower than those at the end of this period. The patent periods reported for the genus *Strongyloides* are found to show considerable variation. Sandground (14) has found the duration of *S. stercoralis* infection to be from 2 to 11 months in dogs and from 2 to 7 months in cats. Faust *et al.* (7) state that infection of the dog with human strains of *Strongyloides* is maintained for only a period of weeks or months, as contrasted with a period of years in man, the natural host. The patent period of *S. ratti* has been shown by Sheldon (15) to range from 51 to 136 days. In the present studies with *Strongyloides*, the period over which eggs have been passed in the faeces has been found to show

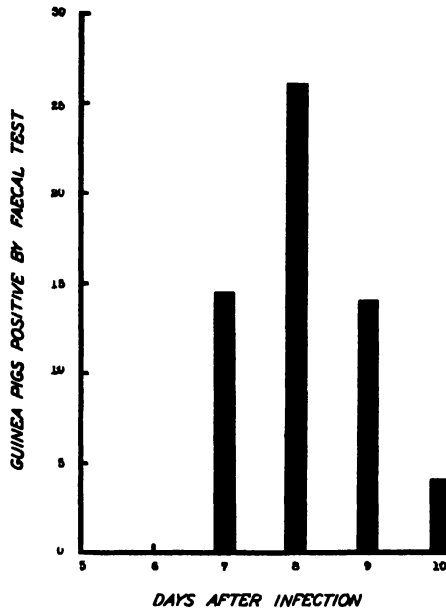


FIG. 4. Frequency distribution of first day of appearance of ova in faeces of 58 guinea pigs after infection with *S. agoutii*.

considerable variation. Eggs were recorded in the faeces of 26 guinea pigs for three to eight weeks. The majority of infections terminated during the fourth or fifth week. A number of individuals were killed subsequent to a negative faecal test, and autopsy showed that the test was a true indication that the infection had disappeared rather than that the females become non-fecund. As the animals were infected with filariform larvae en masse, no data were available to show if the heavier infections were lost more quickly than the lighter ones. It was observed, however, that the older the host at the time of infection, the earlier the infection was lost.

Observations on Migration of Larvae of *S. agoutii* Through the Guinea Pig

It is generally agreed that, following percutaneous infection, the path of migration of *Strongyloides* necessitates the passage of the larvae through the lungs prior to their establishment in the small intestine. To ascertain the rate of migration and to observe the organs in which the various stages were found, four guinea pigs were infected and killed at intervals over a period of eight days.

The animals employed were young males, six weeks old at time of exposure. They were infected en masse with *S. agoutii*, as previously described. Autopsies were made and all suitable organs examined by the Baermann technique to obtain any migrating larvae. In each case, the autopsy included the examination of the trachea, lungs, liver, heart, spleen, kidneys, stomach, small and large intestines.

The first guinea pig was killed 48 hr. after exposure and one filariform larva was obtained in the heart. The second was killed after 72 hr.; a few larvae were observed from the lungs, heart, stomach, duodenum, and jejunum. The third was killed after 96 hr. and yielded filariform larvae from the heart and stomach; larvae in stages of metamorphosis between the filariform larva and the adult were found in the duodenum and jejunum. The fourth was killed on the eighth day, and one filariform larva was obtained from the heart; the duodenum and jejunum yielded large number of both immature and ovigerous females, and a few were also found in the ileum.

As a result of these infections, it appears that with this species the migration through the lungs is of a transitory nature; no forms other than filariform larvae were observed in that organ. The time of migration through the blood stream extends over a considerable period of time, since a larva was collected from the heart as late as the eighth day subsequent to infection.

Acquired and Age Resistance of the Guinea Pig to Infection with *S. agoutii*

The subject of resistance and immunity of the host to infection with helminths has received but little attention until recent years. From the point of view of the host there are three major types of resistance mechanism. The first type is natural immunity, which is found frequently among animals. Most individuals are found to be naturally immune to parasites of unrelated animals, though wide adaptations are known to exist. The second type is that of age resistance. This is displayed by animals that may be susceptible in early life to infection with a parasite, but become less susceptible later. The third type is that which is acquired by the host as a direct consequence of infection with a specific parasite.

Previous work on resistance to re-infection among members of the genus *Strongyloides* is scanty, and Sandground (14) appears to have been the first to make studies of this nature. He reported investigations on susceptibility, resistance, and acquired immunity of dogs and cats to infection with *S. stercoralis*. Successful infection of dogs and cats of all ages was brought about with a human strain of *S. stercoralis*, and animals that lost their infection were found to be refractory to re-infection; the acquired immunity in dogs lasted for more than six months. It was concluded that in immune animals the larvae reach the intestine but do not attain sexual maturity. Kotlan and Vajda (11) report an age immunity to *S. ransomi* occurs in pigs. Sheldon (16) carried out quantitative studies on acquired resistance and showed that a marked resistance was acquired by rats as the result of super-infection with *S. rattii*. He also demonstrated that resistance was acquired and retained by rats as the result of a single previous infection that had run its course and disappeared; they were also found to be successfully immunized by serial injections of heat-killed larvae in a saline suspension. Animals two or four months old were found to be equally susceptible to infection with *S. rattii*;

at eight months resistance was observed, at 12 months was less apparent, and was observed again at 17 months.

A series of experiments was undertaken to determine if an acquired or an age resistance was manifested by guinea pigs to *S. agoutii*. If an acquired resistance was exhibited, it was hoped to discover whether this tended to pass off after a time or if it inhibited reproduction of the female or prevented the parasite from establishing itself in the intestine with the resultant expulsion of the worm in the faeces.

A total of 17 guinea pigs was used. Several of these had been used in the experiments on serial transfer, thus providing excellent material on which to attempt re-infection with the same parasite. Infection of the guinea pigs was effected percutaneously, as described previously. Diagnosis of infection was determined by faecal test.

The age of animals employed in these tests varied to a considerable extent. Control guinea pigs were employed to check the infectivity of larvae used for re-infection trials. Post-mortem examinations were not made on all guinea pigs as some were used for other purposes. In certain instances when negative faecal tests were recorded, autopsy was carried out to ascertain if females were present but had been rendered non-fecund by an immunity mechanism. In no case was this found to occur.

The essential data relating to this series of infections are presented in the accompanying tables. To determine if any resistance existed, a preliminary trial was initiated, the results of which are presented in Table I. Three guinea pigs had previously been infected with *Strongyloides*. Prior to the second exposure they were shown by faecal test to have eliminated their primary infection. This exposure was about six months after the first; they did not become re-infected. A third exposure was made one month after the second but no evidence of infection was observed. No autopsy was carried out.

The second group comprised four individuals previously infected and two controls (Table II). These four animals gave a negative faecal test for several months before re-infection was attempted. Since guinea pig No. 4 was exposed to larvae from a different culture to that used as a source of larvae for Nos. 5, 6 and 7, two young controls were exposed to ensure the infectivity of both lots of larvae. The guinea pigs were all exposed to infection on the same day, and subsequent faecal tests yielded negative results for Nos. 4-7; both controls were positive. Chandler (4) has shown that a resistance acquired as a result of a previous infection of *Nippostrongylus muris* in rats may materially interfere with reproduction of the parasite and cause a pronounced reduction in egg output. In view of this fact it was considered advisable to autopsy Nos. 4-7, to make sure that the routine faecal tests were not yielding inaccurate results. Nos. 5 and 6 were autopsied 14 and 13 days after infection, Nos. 4 and 7, 31 days after infection. Thorough examination of the lungs, heart, liver, small and large intestines did not yield any stages of *Strongyloides*. The control to No. 4 was killed and examined on the 23rd

TABLE I
PRELIMINARY TRIAL OF EXPOSURE OF GUINEA PIGS TO RE-1 INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos., 1937	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure		Date of 3rd expos., 1938	Faecal tests after 3rd exposure	
			Date	Result		Date	Result		Date	Result
1	4½	June 17	June 25, 26, 28, 29 July 15 Aug. 16 Oct. 13, 27 Dec. 1, 21 Jan. 1	Positive Positive Positive Negative Negative Negative	Jan. 27	Feb. 3-5, 9-11, 15, 22, 25, 28	Negative	Mar. 2	Mar. 9-12, 14, 15, 22	Negative
2	5	July 15	July 24, 26, 27 Aug. 5, 16 Oct. 10, 26 Dec. 1, 21 Jan. 12	Positive Positive Negative Negative Negative	Jan. 27	Feb. 3-5, 9-11, 15, 17, 19, 22, 25	Negative	Mar. 2	Mar. 9-12, 14, 15, 22	Negative
3	2	July 27	Aug. 5, 6, 16 Oct. 13, 19, 27 Dec. 12 Jan. 1	Positive Negative Negative Negative	Feb. 2	Feb. 9, 10, 15, 17, 20, 22, 25, 28	Negative	Mar. 2	Mar. 10-12, 14, 15, 24	Negative

TABLE II
EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos., 1938	Faecal tests after 1st exposure		Date of 2nd expos., 1938	Faecal tests after 2nd exposure	
			Date	Results		Date	Results
4	12	April 1	April 9-11, 13, 19, 27 May 16, June 6, 20 July 18, Aug. 9, Oct. 13, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12-17, 19, 20, 27	Negative
Control	1½	Dec. 5	Dec. 12-17, 19, 20	Positive			
5	2	Jan. 10	Jan. 19, 20, Feb. 3, 17 Feb. 22, 28, Mar. 22 April 4, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 13, 17, 24	Positive Negative Negative Negative Negative	Dec. 5	Dec. 12, 14, 15, 17, 18	Negative
6	2	Mar. 10	Mar. 17, 18, April 5 April 19, May 5 June 6, 20, July 18 Aug. 8, Oct. 13, 17, 20	Positive Negative Negative Negative	Dec. 5	Dec. 12-16	Negative
7	2	Mar. 29	April 4, 6, 19, May 16 June 6, 20, July 18 Aug. 9, Oct. 10, 17, 24	Positive Negative Negative	Dec. 5	Dec. 12-20, 27	Negative
Control	2	Dec. 5	Dec. 14-17, 19	Positive			

POST-MORTEM EXAMINATION

- No. 4. Killed Jan. 5, 1938. All organs (lungs, heart, liver, duodenum, jejunum, ileum, large intestine) negative for *Strongyloides*.
 Control. Killed Dec. 28, 1938. Small intestine heavily infected with adult *Strongyloides*.
 No. 5. Killed Dec. 19, 1938. All organs negative for *Strongyloides*.
 No. 6. Killed Dec. 18, 1938. All organs negative for *Strongyloides*.
 No. 7. Killed Jan. 5, 1939. All organs negative for *Strongyloides*.

day and displayed a heavy infection of *Strongyloides* in the small intestine; the other control guinea pig was used for further experimentation.

To check these results another experiment was undertaken in which three previously infected, and two control, animals were employed. The essential data on this trial was summarized in Table III. Before attempting infection for the second time, the guinea pigs were shown to have been negative to faecal tests over a period of some months. One control was employed to test the infectivity of larvae used for application to Nos. 8 and 9, another for No. 10. After attempting infection, these animals received the usual routine faecal examinations, negative results being recorded in all cases. Nos. 8 and 9 were autopsied on the 9th and 11th days after exposure; the control was killed on the 8th day. No autopsy of No. 10 and the control was carried out since they were held for further use. Post-mortem examination of Nos. 8 and 9 did not reveal any stages of *Strongyloides* in the intestine, whereas one larva was recorded from the heart of the control, and the small intestine was heavily infected with adults.

TABLE III
EXPOSURE OF GUINEA PIGS TO RE-INFECTION WITH *Strongyloides agoutii*

Guinea pig No.	Age at 1st expos., months	Date of 1st expos.	Faecal tests after 1st exposure		Date of 2nd expos., 1939	(F) Faecal and (B) Baermann* tests after 2nd exposure	
			Date	Results		Date	Results
8	2	Dec. 20 1937	Dec. 30, Jan. 17 Feb. 3, 20, 22, Mar. 15, 22 April 5, 19, May 16 June 6, 20, July 18 Oct. 13, 17, 24	Positive Negative Negative Negative Negative	Jan. 9	(F) Jan. 16-18 (B) Jan. 10-18	Negative Negative
9	2	May 13 1938	May 20-22, June 18, 20 Aug. 8, Oct. 13, 17, 25	Positive Negative	Jan. 9	(F) Jan. 16-20 (B) Jan. 10-20	Negative Negative
Control	1½	Jan. 9 1939	Jan. 16	Positive	Jan. 9		
10	2	June 2 1938	June 10, 13, 20, July 18 Aug. 9, Oct. 13, 17, 25	Positive Negative	Jan. 30	(F) Feb. 7, 9-11, 13-15, 20, 23 (B) Jan. 31-Feb. 8	Negative Negative
Control	1½	Jan. 30 1939	Feb. 7-9, 10, 13, 20, 27 Mar. 21	Positive Negative			

* Baermann examination was made in morning and late afternoon each day.

POST-MORTEM EXAMINATION

No. 8. Killed Jan. 18, 1939. All organs negative for *Strongyloides*.
 No. 9. Killed Jan. 20, 1939. All organs negative for *Strongyloides*.
 Control. Killed Jan. 17, 1939. 1 larva in heart. Intestine heavily infected with adult *Strongyloides*.

From Table III it will be seen that for the first 8 to 10 days following the second exposure to infective larva, a Baermann examination was introduced in addition to the routine faecal test. When an acquired resistance is manifested by an individual, there appears to be a generalized condition which may inhibit development to the adult stage or prevent successful establishment of the invading parasite within the intestine. In view of this fact and on the assumption that the migrating infective larvae would, if they arrived at the intestine, still be active, collection of the total faecal output of Nos. 8, 9, and 10 was made twice daily and Baermanned, the sediment being drawn off after one-half and one hour. It was anticipated that if the migrating larvae did not become established in the intestine they would be passed out, but no larvae were found.

Since a number of the individuals employed in the preceding trials were, on an average, about one year old at time of re-exposure, an attempt was made to determine whether this resistance was due to an age factor. Three guinea pigs known to be considerably more than a year old were used for this purpose, the data on which are recorded in Table IV. All three were

TABLE IV

INFECTION OF ADULT GUINEA PIGS WITH INFECTIVE LARVAE OF *Strongyloides agoutii*

Guinea pig	Date exposed, 1938	Faecal tests	
		Date	Results
A	Dec. 29	Jan. 7-10, 12, 16, 18, 20, 23, 25, 30 Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
B	Dec. 29	Jan. 7-10, 12, 16, 20, 23, 25 Jan. 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative
C	Dec. 29	Jan. 7-10, 12, 16, 18, 20, 23 Jan. 25, 30, Feb. 4, 6, 13, 20, 27, Mar. 23	Positive Negative

infected on the same day and faecal tests throughout the month following exposure were all positive. The infection was observed to pass off after the first month; these animals were not autopsied but were held for further use.

The preceding experiments show that the guinea pig acquires a resistance to re-infection with *Strongyloides*. This resistance does not permit the establishment of the parasite in the intestine, and animals that have lost their infections cannot be re-infected over a period ranging at least from 6 to 13 months after the primary infection. An attempt was made to ascertain whether infective larvae were expelled from the intestine with the faecal material; results of observations were negative but because of complicating factors these observations are not considered conclusive.

Acknowledgments

It is with very much pleasure that the author expresses his sincere appreciation and thanks to Professor T. W. M. Cameron for his guidance and generous assistance in the direction of these studies.

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THE HELMINTH PARASITES OF SLEDGE-DOGS IN NORTHERN CANADA AND NEWFOUNDLAND¹

BY THOMAS W. M. CAMERON², I. W. PARNELL³, AND L. L. LYSTER⁴

Abstract

In a survey, based on examination of faeces and viscera of sledge dogs, the following were identified: hookworms, ascarids, whipworms, kidney worms, fish-carried and other tapeworms, the Canadian liver-fluke and other trematodes and an acanthocephalid. The distribution of the infections is noted. The acanthocephalid, *Corynosoma semerme*, is recorded for the first time from North America.

Since 1933, the Institute of Parasitology has been engaged in a survey of the parasites of animals in Canada. During that period it has accumulated a considerable amount of data on infections of economically important animals in the Arctic and sub-Arctic regions.

The parasitic fauna of animals is of considerable importance to the inhabitants of these regions. The aboriginal peoples live entirely on the animals that breed there and although of an advanced culture, many, particularly Esquimaux, eat a large part of their meat and fish uncooked. Because of the rigour of the climate, human beings and domestic animals live close together and make the transmission of helminths a simple matter. Because of the severe climate, bacterial infections are somewhat rare and animal parasites have become of even greater significance.

The animal of greatest importance is the dog. Transportation and communication of white man and native, alike, depend almost exclusively upon this animal. Consequently an investigation of the parasites of this animal has been a first consideration. The data at our command are now sufficient to record and give an adequate picture of the geographical distribution of its parasites in these regions.

The information was mostly obtained through co-operation of the R.C.M. Police, the National Parks Branch, and the Hudson's Bay Company in Canada, and of the Newfoundland Rangers in Labrador. In addition, both junior authors have personally collected in the eastern Arctic, one (I.W.P.) in 1933 in the north and west sides of the Quebec Peninsula, and the other (L.L.L.) in 1939 from that region and from Baffin, Ellesmere, Southampton and Somerset Islands, and the west coast of Hudson's Bay.

Much information has been obtained by examination of faeces sent to the Institute by interested persons, including the R.C.M. Police, Game Wardens,

¹ Manuscript received April 4, 1940.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

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Hookworms have been found in dogs in all areas surveyed and they occur in the most northerly station in North America, viz., Craig Harbour, Ellesmere Island, N.W.T. In many cases, the diagnosis has been based only on eggs. As, however, only the northern dog hookworm (*Uncinaria stenocephala*) has been found (on autopsy) north of Moosonee, it is probable that all these records refer to this species.

Egg records are as follows—

Arctic Red River, Mackenzie District, N.W.T.

Battle Harbour, Labrador.

Bonne Baie, Nfld.

Cambridge Bay, Victoria Island, N.W.T.

Cartwright, Labrador.

Chimo, Ungava Bay, Que.

Craig Harbour, Ellesmere Island, N.W.T.

Flower Cove, Nfld.

Fort Fitzgerald, Alta.

Fort George, Man.

Hebron, Labrador.

Hopedale, Labrador.

Lake Harbour, Baffin Island, N.W.T.

Maitland Point, Mackenzie District, N.W.T.

McLeod Lake, B.C.

Moosonee, James Bay, Ont.

Nain, Labrador.

Northwest River, Labrador.

Pangnirtung, Baffin Island, N.W.T.

Pine River, Man.

Port Burwell, Hudson Strait, N.W.T.

Port Harrison, Hudson Bay, Que.

Stupart Bay, Hudson Strait, Que.

William Harbour, Labrador.

Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, this species has been found in carcasses from Dundas, Moosonee, Burwell, Sept Isles, and Nipigon. Two dogs were positive at Dundas; of these one was born on Devon Island while the other had been imported from Pangnirtung on Baffin Island. This species also occurs in dogs on the Island of Montreal, P.Q., and is common in foxes throughout Eastern Canada.

Ancylostoma caninum, the southern dog hookworm, was found in one dog from Moosonee, in association with the northern species. This appears to be the most northerly record of its occurrence. It also occurs on the Island of Montreal, P.Q.

Ascarids

Ascarids are almost equally widely spread, the predominant species being *Toxascaris leonina* (syn. *T. felis*) although *Belascaris marginata* (syn. *Toxocara canis*) also occurs; these species were not separated in the writers' egg records, although most were *T. leonina*.

Egg records were obtained from:

Cartwright, Labrador.

Chimo, Ungava Bay, Que.

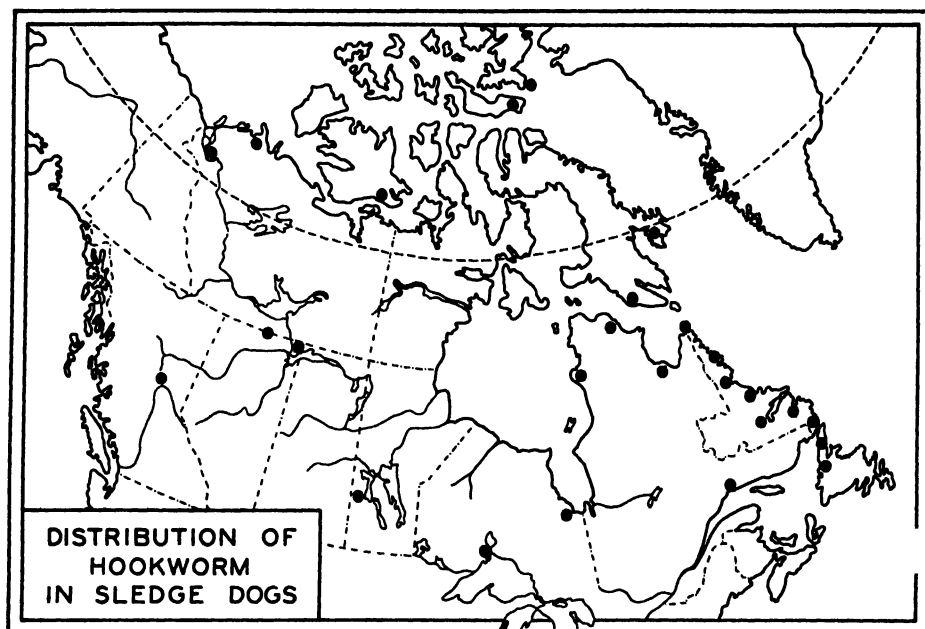


FIG. 2

Churchill, Man.

Cumberland House, Sask.

Eskimo Point, Keewatin District, N.W.T.

Fort Chipewyan, Alta.

Fort Resolution, Mackenzie District, N.W.T.

Hebron, Labrador.

Hopedale, Labrador.

Lake Harbour, Baffin Island, N.W.T.

Maitland Point, Mackenzie District, N.W.T.

Moosonee, James Bay, Ont.

Nain, Labrador.

Rupert House, James Bay, Que.

William Harbour, Labrador.

Wood Buffalo Park, Mackenzie District, N.W.T.

In addition, actual specimens of *T. leonina* from dogs were collected from Cape Smith, Dorset, Moosonee, Nottingham, Stupart and Wolstenholme, and of *B. marginata* from Moosonee and Wolstenholme.

Whipworms

Whipworms (*Trichuris vulpis*) were seen on only one occasion (Kenora, Ont.) and were not recovered from Arctic regions at all. They are fairly common in southern Canada.

Kidney Worm

A single infection of *Dioclophyme renale* was recorded from Berens. In this case both kidneys were infected and the female worms reached a length of 30 in. The dog died as a result of this infection.

Tapeworms

Dipyllobothrium spp. At least two species of this genus appear to occur in dogs in the north of Canada. One, which is found in the Mackenzie River basin, is associated with fresh-water fish and is almost certainly *D. latum*. Most records were based on eggs in the faeces, but actual tapeworms found in man in northern Saskatchewan belong to this species, and the distribution is continuous from this region.

The other species, occurring in northern Quebec and along the Arctic coast, has a longer egg and the carrier fish may be assumed to be a salt-water one. The few specimens recovered from dogs in that region were in too poor a state of preservation for proper identification and could be referred neither to an existing species nor a new one. Accordingly, while the writers believe that at least two species exist, they have not attempted, at this stage, to identify them by name.

Eggs of tapeworms of this genus were found in the following places:

Arctic Bay, Baffin Island, N.W.T.

Baillie Island, N.W.T.

Berens, Man.

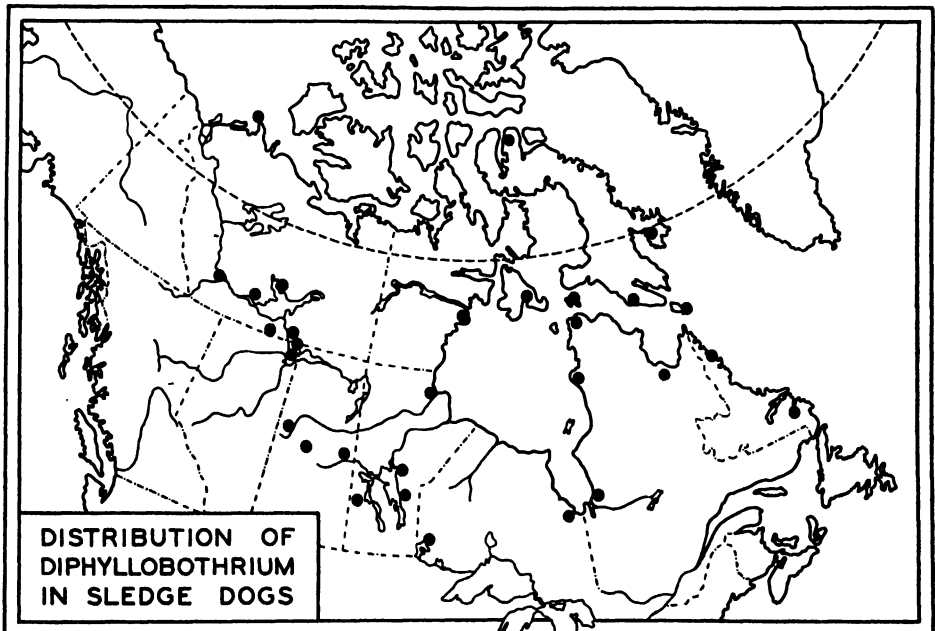


FIG. 3

Cameron Bay, Keewatin District, N.W.T.
Cartwright, Labrador.
Cat Lake, Ont.
Chesterfield, Keewatin District, N.W.T.
Chimo, Ungava Bay, Que.
Churchill, Man.
Coral Harbour, Southampton Island, N.W.T.
Cumberland House, Man.
Forteau, Labrador.
Fort Chipewayan, Alta.
Fort Fitzgerald, Alta.
Fort Providence, Mackenzie District, N.W.T.
Fort Smith, Mackenzie District, N.W.T.
Goldfields, Man.
Hebron, Labrador.
Ile a la Crosse, Sask.
Kenora, Ont.
Lake Harbour, Baffin Island, N.W.T.
Moosonee, James Bay, Ont.
Norway House, Man.
Pangnirtung, Baffin Island, N.W.T.
Pine River, Man.
Port Harrison, Hudson Bay, Que.
Rae, Mackenzie District, N.W.T.
Resolution, Hudson Strait, Que.
Rupert House, James Bay.
Simpson, Mackenzie District, N.W.T.
Waskesiu Lake, Sask.
William Harbour, Labrador
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens referable to this genus were found in Wolstenholme and Nottingham Island.

Taenia spp.

Five dogs showed *Taenia* eggs in their faeces, but again it was impossible to refer them to a species. These cases came from:

Cameron Bay, Keewatin, N.W.T.
Churchill, Man.
MacLeod Lake, B.C.
Pine River, Ont.
Rupert House, James Bay, P.Q.

Specimens of *Taenia*, not identifiable because of partial decomposition, were found in Notre Dame du Nord, Que., and Cape Smith.

Dipylidium caninum

This tapeworm was found in carcasses from Moosonee, Nottingham and Wolstenholme.

Trematodes*Metorchis conjunctus*

The Canadian liver fluke was found in dogs from:

Berens, Man.
Cat Lake, Ont.
Cumberland House, Sask.
Ile a la Cross, Sask.
Kenora, Ont.
Lac la Ronge, Sask.
Lake Mistassini, Que.
Moosonee, James Bay, Ont.
Norway House, Man.
Pine River, Ont.
Rupert House, James Bay, Que.
Trout Lake, Ont.
Waskesiu Lake, Sask.

The southern limit of this parasite appears, from present records, to be the St. Lawrence and the Canadian border. Its northern limit in Quebec is unknown, but its western and northern limits appear to be the height of land in Saskatchewan and the borders of the N.W.T.

Cryptocotyle lingua

This intestinal trematode, which appears to have been introduced to the Maritime Provinces of Canada, has spread both north and south. It is now found in the St. Lawrence estuary and the Labrador.

Records from the area under review include:

Anticosti Island, Gulf of St. Lawrence.
Cartwright, Labrador.
Flower Cove, Nfld.
Hebron, Labrador.
Hopedale, Labrador.

Alaria sp.

A species of Strigeid, which on the morphology of the egg and its resemblance to that found by us in actual trematodes, we refer to this genus, is recorded from:

Cumberland House, Sask.
Moosonee, James Bay, Ont.
Rupert House, James Bay, Que.
Wood Buffalo Park, Mackenzie District, N.W.T.

Actual specimens of a species of *Alaria* were recovered from a dog from Moosonee.

Miscellaneous Trematodes

Trematode eggs, which the writers were unable to identify because of their non-correspondence to published descriptions or to specimens collected from these regions, occurred in the faeces of dogs from:

Berens, Man.

Cambridge Bay, N.W.T.

Eskimo Point, N.W.T.

Fort Smith, N.W.T.

Port Harrison, Que.

Acanthocephala

A single species of *Acanthocephala* was recovered from a husky dog at Dundas, N.W.T. It has been identified by Prof. H. J. Van Cleave as *Corynosoma semerme* (personal communication), a species hitherto unrecorded from North America.

A NEW TREMATODE, *FIBRICOLA LARUEI*, FROM THE RACCOON IN CANADA¹

BY M. J. MILLER²

Abstract

A new trematode, *Fibricola laruei*, is described from the raccoon in Canada.

The raccoon (*Procyon lotor*) in the Province of Quebec was found to harbour an undescribed strigeid of the sub-family Alariinae. Its morphology showed it to be most closely related to members of the genus *Fibricola*, to which it is assigned, with the specific designation *laruei* sp. nov.

Description

Small, comparatively thin forms measuring from 0.7 to 1.17 mm. in length when mature; body divided into two parts, the lengths of the anterior and posterior parts being in the approximate ratio of 3 : 2; anterior part of body flattened with the edges inrolled ventrally and meeting medially near the junction of the two segments of the body (0.46 to 0.5 mm. wide); posterior segment narrow and cylindric (0.22 to 0.3 mm. wide); terminal oral sucker slightly longer than broad (average size, 0.076 by 0.066 mm.); pharynx conspicuous, elongate, about the same length as the oral sucker and about two-thirds as broad; oesophagus slightly shorter than pharynx; intestinal caeca extend well into the posterior segment; ventral sucker usually broader than long, situated at the junction of the anterior and middle thirds of the body (average size, 0.026 by 0.033 mm.); muscular holdfast organ situated medially at the posterior end of the anterior segment (average size, 0.11 by 0.07 mm.); vitellaria composed of numerous small follicles confined to the anterior segment; testes two in number situated asymmetrically in the posterior segment, the posterior testis considerably broader, reniform in shape, the anterior testis situated immediately above the posterior one usually on the right, but occasionally on the left side of the body, roughly spherical in shape (average size posterior testis, 0.06 by 0.17 mm.; average diameter anterior testis, 0.13 mm.) ovary small, wider than long, above and partially overlapping anterior testis in a dorsal position (average size, 0.08 by 0.05 mm.); eggs large, rarely over two present in the uterus at one time (average size, 0.12 by 0.07 mm.).

Discussion

According to Dubois (2) the subfamily Alariinae contains five genera: *Alaria*, *Cynodiplostomum*, *Fibricola*, *Pharyngostomum*, and *Podospathalum*. The present form obviously does not belong to either *Pharyngostomum* or

¹ Manuscript received March 4, 1940.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada, awarded through the National Committee on Fish Culture.

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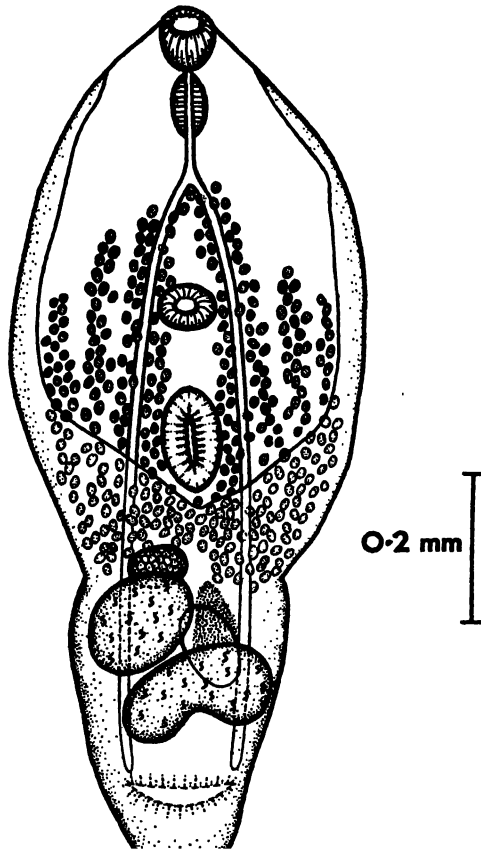


FIG. 1

Podospathalum. It differs from *Alaria* and *Cynodiplostomum* in that it has no pseudo-suckers or other appendages of any sort at the cephalic extremity. It differs from *Fibricola* in the character of the holdfast organ, which is elliptical in the present form and circular in *Fibricola*. With the exception of this character, however, its general morphology agrees very closely with that of the *Fibricola* species. The difference in shape of the holdfast organ does not appear to be sufficient reason for the creation of a new genus, and so the present form is assigned to the genus *Fibricola* and the generic definition amended to read as follows:

Alariinae with the body more or less distinctly divided into two parts; the cephalic extremity without sucker-like or other appendages; the holdfast organ either circular and one-third of the length of the anterior segment or elliptical and about one-fifth the length of the anterior segment; the posterior segment shorter than the anterior one; the ovary situated at the junction of the two segments; the posterior testis larger than the anterior one, which is asymmetrically developed opposite the Mhelis gland; no genital cone; shallow *burs copulatrix* with a subterminal pore.

Host: Raccoon (*Procyon lotor*)

Location: Small intestine.

Locality: Argenteuil Co., Quebec, Canada. (It has also been found in raccoons from other parts of Quebec.)

Types and paratypes in the helminthological collection, Institute of Parasitology, McGill University, Macdonald College, P.Q., Canada.

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A REVISION OF THE AMERICAN SPECIES OF *GONIA* MEIGEN (DIPTERA: TACHINIDAE)¹

BY FRANK O. MORRISON²

Abstract

Twenty American species of *Gonia* are separated on the basis of the shape of the anal forceps coupled with other characters. Four species, *G. grandipulvilli*, *G. discalis*, *G. albagenae*, and *G. tenuisforceps*, are described as new. The anal forceps are figured for each species. *G. brevipulvilli* Tothill is a synonym for *G. longisforceps* Tothill. A phylogenetic order and grouping of the species is based on the shape of the forceps, season of capture, and habitat. Complete taxonomic history of all categories is given.

Introduction

The tachinid genus, *Gonia* Meigen, has long been known to contain several distinct American species. Descriptions of at least 19 of these have been published. Determinations have been based on many different characters, of which those of the male genitalia have been favoured recently. It is, however, admittedly difficult, if not almost impossible, to describe in words slight differences in the shape or contour of such structures. Yet in no case has one of these characters been illustrated. As a result the accurate specific determination of *Gonia* has been practically impossible, and in almost all dipterous collections various species of this genus may be found under the name of *Gonia capitata* DeGeer, a European species of which the occurrence in America is very doubtful.

The recent extensive work on biological control has brought into prominence our various entomophagous parasites, and especially those parasitic on pests of economic importance. *Gonia* is known to be parasitic on lepidopterous larvae and has been held (17) to be an important control factor in damaging cutworm outbreaks in the grain-growing areas of Western Canada.

Townsend (21), discussing the habits of this and related insects that lay their microtype eggs on plant foliage, where they are ingested with the foliage by lepidopterous and other larvae, goes so far as to suggest that such parasites might be bred and distributed as a control measure for certain pests.

A clarification of the taxonomy of this group has been attempted in the present work by a study of the genital and other significant distinguishing characters. Where possible the work has been based on an examination of type material. A complete taxonomic bibliography of the genus and each species dealt with is included. Some synonymy is suggested, but it seems possible that, when further type material is available and especially when

¹ Manuscript received January 19, 1940, and as revised May 9, 1940.

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 138. Based on a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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European forms can be studied, further synonymy may be shown. Four new species are described and figured.

In approaching this problem an anatomical study of the male genitalia of *Gonia* was found imperative. Such a study forms the second part of this paper, to be published later. It was hoped that less easily exposed structures than those previously used might prove valuable in taxonomic work. Although no characters of taxonomic value were found, it became evident from the literature on the morphology of the male genitalia of Calyptrate Diptera that, though extensively used for taxonomy, the morphology of these structures is as yet poorly understood. The literature was carefully reviewed and synopsized. Considerations of space, however, make it inadvisable to include the synopsis. A table of synonymous morphological terms compiled from the literature is included, as are also the citations of the papers used and a detailed account of the anatomical study of the genital structures in *Gonia*, with some suggestions as to the possible homologies of the parts with those in other Diptera and with the parts of the male genitalia of more generalized insects. The taxonomy of Diptera in general cannot but be furthered by a more complete understanding of, and more uniform terminology for, the parts of these much used structures.

Historical Review

The genus *Gonia* was established by Meigen (9) in 1803, who later described 13 species (10). Curtis (5) designated *Musca capitata* DeGeer as the type of the genus. Desvoidy (6) described two genera, *Rhedia* and *Reaumuria*, which were synonymized with *Gonia* by Coquillett (3). Desvoidy had wrongly supposed the term *Gonia* preoccupied in conchology. Two other generic descriptions published by Desvoidy (7), *Isomerya* and *Pissemya*, have been synonymized with *Gonia* Meigen by Schiner (14) and Brauer and Bergenstam (2) respectively. Townsend (21), in his as yet only partly published "Manual of Myiology", recognizes as separate genera, *Reaumuria* Desvoidy (type *Gonia ornata* Meigen) and *Rhedia* Desvoidy (type *Gonia atra* Meigen), as well as three American genera: *Cnephalogonia* Townsend (type *Gonia distincta* Smith), *Cystogonia* Townsend (type *Gonia turgida* Coquillett) and *Knabia* Townsend (type *Knabia hirsuta* Townsend), and at least one other exotic genus, *Phosphocephalops* Townsend (type *Gonia pallens* Williston), all of which would fall within *Gonia* Meigen as considered here. *Isomerya* Desvoidy he synonymized with *Gonia* Meigen s.s. and *Pissemya* Desvoidy with *Rhedia* Desvoidy. It is impossible, however, to determine from the volumes published to date the limits of Townsend's restricted genera or their synonymy with species considered in this paper. Further definition of these forms must await the publication of the additional volumes of his work containing the generic and specific descriptions.

Meigen and other European taxonomists depended largely on colour characters and the relative lengths of the arisal segments for specific determinations in this genus. The resulting descriptions are totally inadequate to allow any

comparison of our now known American species with the European forms on the basis of these descriptions alone.

Say (13) described the first known American species, *Gonia frontosa* Say, stressing colour. What is probably the same species was redescribed as *Gonia philadelphia* Macquart (8) and again as *Gonia albifrons* Walker (22). Williston (24) recognized the synonym of the above mentioned names, redescribed the species as *Gonia frontosa* Say, and described four new species, giving a key for the separation of the five species mentioned. He discarded the relative lengths of the arisal segments as a character variable in individuals and noted the colour of the antennae, colour of the thoracic vestiture, and length of the claws and pulvilli in the males.

Townsend (19) added another species, *Gonia sagax* Townsend.

Coquillett (3) reduced the previously described species to two, synonymizing *Gonia sagax* Townsend and *Gonia senilis* Williston, and synonymizing *Gonia frontosa* Say, *Gonia exul* Williston, and the European species *Gonia capitata* DeGeer, and described a third, new species, *Gonia turgida* Coquillett. *Gonia porca* Williston he recorded as "unrecognized".

Smith (15) described a new species, *Gonia distincta* Smith, basing his description on females only. In the following year Townsend (20) described a new, related genus, *Cnephalogonia* Townsend, with *Gonia distincta* Smith as the type species.

Strickland (17), working with cutworm outbreaks, found very different types of larvae of *Gonia* present. Tothill (18) revised the genus, largely on material reared by Strickland. Tothill's was the first classification based on male genitalia, the forceps of the genitalia being described but not figured. He recognized 17 species, 10 of which he described as new. *Gonia frontosa* Say, *Gonia sagax* Townsend, *Gonia senilis* Williston, *Gonia porca* Williston, and *Gonia sequax* Williston, he recognized as distinct species along with *Gonia distincta* Smith and *Gonia turgida* Coquillett.

Reinhard (12) described an additional new species, *Gonia texensis* Reinhard, again describing but not illustrating the male genitalia.

Present Revision of the Genus

The writer commenced work on this genus at the University of Alberta, where a considerable collection of *Gonia* had accumulated. This was augmented by specimens from the University of Montana, the Dominion Entomological Laboratory at Saskatoon, Saskatchewan, and material collected by the writer and others at Macdonald College, Province of Quebec. Dr. McDunnough, Department of Agriculture, Ottawa, kindly allowed the examination of Tothill's type material and of several hundreds of specimens in the Canadian National Collection.

Early in the investigation it became evident that, at least at present, accurate determination of female material must remain impossible. It is the hope of the writer to investigate, at a later date, the connection between the species

as here determined on male characters, and the different types of larvae (17). This, together with a study of the female genitalia, might lead to the discovery of means of classifying females.

For the purpose of the present paper, males only are considered except where the female definitely associated with these males is known. The key is based on combinations of characters of the genitalia and of other structures. The only portion of the male genitalia showing characters of taxonomic value is the anal forceps (Fig. 1). This compound structure is normally folded underneath the abdomen and concealed in the genital pouch beneath the lobes of the fifth sternite. From this position it must be extruded with a hooked needle in order that lateral and dorsal views may be observed. The terms "dorsal" and "ventral" as used in this paper refer to the forceps in this extended position. The outline of the forceps in the dorsal and lateral views offers the best characters for separating the species.

The drawings presented here were prepared from genitalia (of type material where possible) first removed from the specimen, then treated for from 24 to 36 hr. in cold 10% potassium hydroxide, and kept in vials of 40% alcohol and 10% glycerine solution. They were examined in glycerine with the aid of a binocular microscope. A camera lucida was attached to one tube of the microscope to aid in securing the outline. Permanent mounts were made in balsam or De Faure's solution, but this is not advisable as it prevents any further moving of the material. For purposes of determination in later work, it did not prove necessary to dissect out the genitalia from the body. They were merely extruded to reveal the forceps and dried in that position. A word of warning should be given here pointing out that unless the observations are made from perfectly similar positions in all cases (i.e., lateral and dorsal views), appearances may be very misleading. The dorsal view is difficult to get in such a way that the two sides of the forceps are symmetrical. Thus an apparent asymmetry is noticeable in some of the diagrams presented in this paper.

In many species the appearance of the genitalia is not sufficiently distinctive to serve alone as a distinguishing character. Other morphological structures must then be used.

The key given has been built with one chief thought in mind; i.e., that it should be usable and make exact separation of the species possible. For this reason the species key out separately and not into the groups of related species that are discussed later.

Gonia Meigen

1803 GONIA Meigen (9). 1826 GONIA Meigen (10), (13 spp. described). 1830 RHEDIA Desvoidy (6) (Synonymy by Coquillett). 1830 REAUMURIA Desvoidy (7), (Synonymy by Coquillett). 1834 GONIA Meigen (5), (*Musca capitata* DeGeer, designated as type). 1849 GONIA Meigen (22). 1851 ISOMERYA Desvoidy (7), (Synonymy by Schiner). 1851 PISSEMYA Desvoidy (7), (Synonymy by Brauer and Bergenstam). 1862 GONIA

Meigen (14). 1878 GONIA Meigen (11). 1887 GONIA Meigen (24), (Desc. and table of spp.). 1894 GONIA Meigen (16), (Discussed generic limits). 1897 GONIA Meigen (3). 1924 GONIA Meigen (18). 1934 GONIA Meigen (21), (Greatly restricted).

Gonia Meigen is easily separated from other genera of Tachinids by the following combination of characters: head very much inflated (except in *G. distincta* Smith), yellowish except on the occiput and the eyes, pollinose with the front above largely translucent and more than twice as wide as either eye in both sexes (wider in females), the frontal vitta not strikingly darker in ground colour, ocellar bristles strong and curved backward, orbitals present in both sexes, eyes bare, antennae black or yellow, the penultimate arisal segment always more than three times as long as wide, usually a strong bend at the junction of the second and third arisal segments giving the arista a geniculate appearance, facial ridges with only a few bristles at their bases, parafacials with hair or bristles; propleura bare; lower lobes of squamae bare above; infrasquamal setulae absent. It is to be noted that the generic keys (3, 25) would eliminate *Gonia setigera* Tothill, in which the first vein is setose, from this genus. The earlier generic descriptions also list the palpi as yellow but in the *fissiforceps* group specimens show palpi with varying degrees of infuscation to dark brown. Tothill describes the first two antennal segments as yellow in all cases. This is not quite correct as frequently only a slight reddish-yellow colour on the apical portions of these segments is obvious, the segments being otherwise dark.

KEY TO THE MALES OF *Gonia* MEIGEN FOUND IN AMERICA NORTH OF MEXICO

(For the purposes of this key the genitalia are considered as extruded and extended until the forceps point backward in the direction of the long axis of the body. The terms dorsal, ventral, lateral, "turned up", and "turned down" refer to the structures in this position. Figs. 1 to 22 are lateral views of the forceps; Figs. 1a to 22a, dorsal views.)

1. First, second, and third segments of antennae entirely yellow, aristae black or mostly yellow.....2.
At least most of the third antennal segments as well as the aristae dark in ground colour.....3.
2. Aristae mostly yellow, genal hairs white; forceps thick, straight on the ventral edge when viewed in profile, not over twice as long as the median width; as in Figs. 13, 13a.....(No. 13) *senilis* Williston.
Aristae black, genal hairs dark; forceps three times as long as their median breadth, ventral edge convex toward the apex, as in Figs. 2, 2a.....(No. 2) *sagax* Townsend.
3. First wing vein bare with at the most one or two setae.....4.
First wing vein setose on the dorsal side, basally, half way to the first cross vein; forceps as in Figs. 10, 10a.....(No. 10) *setigera* Tothill.

4. Forceps without a conspicuous dorsal carina.....5.
 Forceps with a strongly developed carina as deep as the forceps are thick
 and extending for $\frac{3}{4}$ the length of forceps....(No. 21) *carinata* Tothill.
5. Pile on the occiput white.....6.
 Pile on the occiput brown; forceps as in Figs. 3, 3a.....
 (No. 3) *fuscicollis* Tothill.
6. Pleurae without yellow pile; lobes of fifth sternite black; genal pile light
 or dark.....7.
 Pleurae with yellow pile; lobes of fifth sternite yellow; genal pile fine and
 yellow; forceps as in Figs. 16, 16a.....(No. 16) *porca* Williston.
7. Lobes of the fifth sternite excised laterally (as in Fig. 18b): abdomen
 shining black, with dorsal segmental bases narrowly or not at all
 pollinose.....8.
 Lobes of the fifth sternite not excised laterally; abdomen frequently
 reddish on the sides, dorsal segmental bases often broadly pollinose...9.
8. Forceps more than half as deep as wide at the base of the apical cleft,
 ventral edge almost straight, dorsal convexity extending only about
 one-third of the distance to the apex, which is blunt; in dorsal view
 with the apical cleft extending beyond the middle, as in Figs. 18,
 18a.....(No. 18) *fissiforceps* Tothill.
 Forceps at the base of the apical cleft more than half as deep as wide,
 ventral edge almost straight but the dorsal convexity extending almost
 one-half the length of the forceps, apex blunt, apical cleft not extending
 beyond the middle, as in Figs. 20, 20a....(No. 20) *yukonensis* Tothill.
 Forceps at the base of the apical cleft not half as deep as wide, ventral
 edge curved ventrally at the apex, which is almost hook-like, as in
 Figs. 19, 19a.....(No. 19) *tenuiforceps* new species.
9. Forceps almost as deep as long, dorsally with a subapical tuft of long
 black setae, ventrally with a cushion of yellowish setae in the hollow
 between the apical lobes, as in Figs. 17, 17a..(No. 17) *texensis* Reinhard.
 Forceps much longer than deep, no cushion of yellowish setae between
 the apical lobes ventrally.....10.
10. Genal hairs light in colour.....11.
 Genal hairs dark in colour.....12.
11. Forceps broad as in Fig. 12a, ventral edge slightly curved dorsally at the
 apex as in Fig. 12, abdomen mostly yellow with a narrow dorsal dark
 stripe; abdominal venter yellow except at the base and apex; eastern
 species.....(No. 12) *sequax* Williston.
 Forceps narrow as in Fig. 9a, ventral edge straight as in Fig. 9, abdomen
 mostly yellow with dark dorsal stripe prominent; venter with a longi-
 tudinal black stripe.....(No. 9) *albagenae* new species

12. Parafacials narrower than the greatest width of the eye; pulvilli and unguis long as in Fig. 14b; forceps with dorsal convexity reaching almost to the apex, as in Figs. 14, 14a. (No. 14) *distincta* Smith.
13. Parafacials unusually wide with numerous long black setae next to the eyes and directed medially, setal vestiture of front, parafacials, and genae generally denser, longer, and darker than in any other species; forceps with a straight ventral edge, dorsal convexity not conspicuous, as in Figs. 11, 11a. (No. 11) *turgida* Coquillett.
- Parafacials as usual, with scattered black setae mostly short; forceps with ventral edge curved or if straight forceps are short or have a conspicuous dorsal convexity. 14.
14. Forceps short with a straight ventral edge, with or without a strong dorsal convexity, rarely with the apex slightly dilated dorsally in *breviforceps*. 15.
- Forceps short with a strongly curved ventral edge or longer with a very slightly to strongly curved ventral edge, the apex in profile always dilated dorsally, knob-like. 16.
15. Forceps short, ventral edge straight, dorsal convexity conspicuous and extending about four-fifths of the distance to the apex, as in Figs. 15, 15a; pulvilli as long as the last tarsal segment; unguis slender, as long as pulvilli, yellowish basally, as in Fig. 15b.
- (No. 15) *longipulvilli* Tothill
- Forceps short, and small, ventral edge straight, apex rarely slightly dilated dorsally, as in Figs. 8, 8a; pulvilli and unguis distinctly shorter than the last tarsomere. (No. 8) *breviforceps* Tothill.
16. Forceps 2 to 4 times as long as deep, ventral edge strongly curved, apex distinctly knob-like as in Figs. 1, 1a, small dark species; 7 mm. to 10 mm. long; sides of abdomen sometimes reddish.
- (No. 1) *frontosa* Say.
- Forceps 5 to 8 times as long as deep, ventral edge almost straight to strongly curved, larger species; 9 mm. to 12 mm. long. 17.
17. Forceps in dorsal view triangular, tapering from the base to the apex, as in Figs. 4, 4a; pulvilli and unguis shorter than the last tarsomere, as in Fig. 4b. (No. 4) *aldrichi* Tothill.
- Forceps in dorsal view continuing wide from the base or expanding, then tapering rapidly near the apex, as in Figs. 5, 5a; pulvilli and unguis longer than the last tarsomere, as in Fig. 5b.
- (No. 5) *grandipulvilli* new species.
- Forceps in dorsal view tapering rapidly near the base, then extending long and narrow with almost parallel sides to the apex, as in Fig. 7a. . 18.
18. Dorsal pile on abdomen long and erect; forceps as in Figs. 7, 7a.
- (No. 7) *discaelis* new species.

Dorsal pile on abdomen short and recumbent; forceps as in *discalis* (Figs. 6, 6a).....(No. 6) *longiforceps* Tothill.
(= *brevipulvilli* Tothill).

1. *G. frontosa* Say

1829 *Gonia frontosa* Say (13). 1840 *Gonia philadelphia* Macquart (8), (Described from Philadelphia). 1849 *Gonia albifrons* Walker (22), (Described from the Hudson's Bay Territory). 1878 *Gonia frontosa* Say (11). 1887 *Gonia frontosa* Say (24), (Redescribed = *G. philadelphia* Macq., = *G. albifrons* Walk.). 1897 *Gonia capitata* (DeGeer) Coquillet (3). (Probably incorrect in synonymizing *frontosa* with the European *capitata*) = *G. philadelphia* Macquart = *G. albifrons* Walker = *G. exul* Williston (Probably wrongly) = *G. sequax* Williston (Probably wrongly). 1905 *Gonia capitata* (DeGeer) (7) (Follows Coquillet in synonymy). 1924 *Gonia frontosa* Say, Tothill (18) (Redescribed—neotype named—no synonymy mentioned—distinguished from *G. sequax* Willst. *G. exul* Willst. not mentioned).

There seems to be little doubt of the synonymy of Say's species with *Gonia albifrons* Walker and *Gonia philadelphia* Macquart. The synonymy with *G. sequax* Williston and *G. exul* Williston suggested by Coquillet and followed by Aldrich seems improbable in view of the original description of *sequax* "with abdomen reddish yellow" and of *exul*, "with claws and pulvilli of male large." The synonymy of this species with the European *G. capitata* (DeGeer) is also doubtful. A specimen in the Canadian National Collection from Europe, and bearing the label *G. capitata* (DeGeer), determined by Bezzi, has very different forceps (Figs. 22, 22a). Williston (24), when he redescribed the species from Minnesota males, appears to have had this species, but the New England, North Carolina, and California material which he mentions was evidently a mixture of species with more red coloration on the abdomen, yellowish genal hairs, etc.

Considering the widespread distribution of this species it is probably the oldest form, phylogenetically speaking.

Type locality: upper Missouri River.

Distribution: widespread throughout Canada and United States.

Neotype: No. 785. Canadian National Collection.

2. *G. sagax* Townsend

1893 *Gonia sagax* Townsend (19). 1897 *Gonia senilis* Williston, Coquillet (3), (Synonymy probably incorrect). 1905 *Gonia senilis* Williston (1), (follows Coquillet). 1924 *Gonia sagax* Townsend (18), (Redescribed), Neotype named from Middlesex County, N.J. (Figs. 2, 2a).

The Tothill neotype of this species was captured in April, in Middlesex County, N.J. Length 10 mm., width 4 mm. Abdominal segments three and four are slightly reddish on the sides; the antennae are all yellow except the arista which are brownish black (a feature that checks with Townsend's original description); genal hairs dark; pulvilli short, small, oval; ungues

not more than two-thirds the length of the last tarsomere; face and frontal vitta strongly yellow pollinose. Tothill has described the forceps as, "of medium length and not blunt as in *G. senilis* Williston, about three times as long as their median width and much narrower across the base of the apical cleft than at the middle."

In the Canadian National Collection is a female specimen from Aylmer, Que., 14, v, 1925, taken by G. S. Walley, with the same colour markings but very small, being barely 8 mm. long and appearing at first glance to be *frontosa* Say.

Type locality: Ames, Iowa.

Distribution: Iowa, New Jersey, Quebec (?).

Neotype: in the Canadian National Collection.

3. *G. fuscicollis* Tot.

1924 *Gonia fuscicollis* Tothill (18). (Figs. 3, 3a)

The only specimen of this species available was the male paratype, La Fayette, Ind. April, 1916 (J. M. Aldrich) in the Canadian National Collection. This specimen, Tothill tells us, bears the same data as the holotype in the United States National Museum. The general appearance of this small dark specimen (9 mm. by 4 mm.) is strikingly like *frontosa* Say as is the ventrally arcuate line of the forceps. The ventral line of the forceps is more convex than in any *frontosa* observed. The occipital pile is sparse, long, and brown.

A female from Rosthern, Saskatchewan, May 4, 1925, (K. M. King), in the Canadian National Collection, has a light brownish yellow occipital pile, but the abdomen is broadly red on the sides, the colour spreading ventrally to leave only a narrow median longitudinal dark stripe.

The occipital pile of the other species though generally white often shows varying degrees of yellowing to dark yellow, especially in specimens reared in captivity and specimens which appear to have been "wet" at some time. Further collecting may prove this species good or doubtful; at present it must be maintained on the strength of the type and paratype.

Type locality: La Fayette, Ind.

Distribution: ?

Type: United States National Museum.

frontosa group

The three above named species have short forceps with arcuate ventral edges and appear to form a closely related group. All specimens examined have been captured in April, May, or early June.

4. *G. aldrichi* Tothill

1924 *Gonia aldrichi* Tothill (18). (Figs. 4, 4a)

Among the many paratypes of Tothill's species *aldrichi* are two distinct species. His own description of the forceps, "unusually robust, being both long and wide. About three times as long as the median width and almost triangular in dorsal view the equal sides tapering from the base to tip. The dorsoventral flattening is very marked," is descriptive of the type, and sufficient to separate out this species. Add to this that the unguis and pulvilli in the male are distinctly shorter than the last tarsomere, and no difficulty in separation from the following species should arise.

Tothill records *aldrichi* as bred by Strickland from *Euxoa ochrogaster* and *Agrotis orthogonia* (Lepidoptera: Noctuidae). Adults are collected in April and May.

Type locality: Coaldale, Alberta.

Distribution: across Canada and the northern United States.

Type: No. 786, Canadian National Collection.

5. *G. grandipulvilli* new species (Figs. 5, 5a, 5b)

Male: length, 9 to 12 mm.; width, 4 to 5 mm. A comparatively large, dark species. Face and front light yellow with a silvery sheen in some lights, front above with a waxy appearance on the sides; lines of the face and front in profile almost straight and meeting at an angle just less than a right angle; parafacials narrower at the oral margin than at the vibrissal base, at the narrowest point wider than the greatest eye width, with an uneven double or triple row of black setae (which vary greatly in size) along the inner edge of the eye and separated from the usual row of graduated bristles along the frontal suture by a bare area. Genae less than one-half the eye height with numerous slender dark setae; antennae blackish with a greyish sheen in some lights; second antennal segment slightly reddish apically, with short dorsal setae, third segment five to seven times as long as the second; pile on the occiput white; palpi yellow. Dorsum of the thorax with five broad, longitudinal pollinose lines; scutellum yellowish at least apically, frequently dark basally, some yellow on the humeri, legs black. Pulvilli and unguis distinctly longer than the last tarsomere; the pulvilli broad and white to yellowish brown; unguis black, heavy, and extended almost at right angles to the tarsomere; tarsomeres somewhat broad and flat; wings clear with but very slight costal infuscation, first vein (R_1) bare above, third vein (R_{4+5}) with a group of setae above and below at the point where (R_{2+3}) and (R_{4+5}) separate.

Abdomen with reddish patches on the sides of segments 2 and 3 (this reddish colour though involving considerable of the abdomen in a few specimens examined is characteristically limited to separate patches of varying size on the segments mentioned). Dorsally the abdominal tergites narrowly

to broadly pollinose basally, the pollen spreading out laterally on the fifth segment to cover the sides. Dorsal vestiture short and semi-erect.

Forceps in lateral view resemble those of *aldrichi*, strong, long, and flattened; ventral edge slightly curved, apical dorsal convexity evident, dorsally with sides parallel or divaricating more than half way to the apex, then tapering rapidly to the blunt point, outline much less nearly triangular than in *aldrichi*.

Type:—Edmonton, Alta. 25/4/23. F. S. Carr, No. 5032 Canadian National Collection, (Genitalia, vial No. 27).

Paratypes:—Edmonton, Alta.: as type (vial No. 25); 2, v, 1937, F. O. Morrison; 4, v, 1937, E. H. Strickland. Lethbridge, Alta.: ? Seamans and Strickland; April 17, 1925,—April 11, 1926, 2 specimens, H. F. Gray; May 3, 1923,—April 18, 1923, H. L. Seamans. Saskatoon, Sask.: April 20, 1937, 3 specimens, A. P. Arnason; 15 iv, 1915, ?; 8, v, 1923,—April 26, 1924, 3 specimens,—22, iv, 1923,—May 11, 1925,—10, v, 1923—April 29, 1924, K. M. King; May 11, 1923,—3, v, 1923, N. J. Atkinson. Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Bozeman, Montana: June 2, (192?), ? (vial No. 53). Toronto, Ont.: iv, 25, 1906. Merivale, Ont.: 3, v, 1938, 2 specimens, G. E. Shewell. Maple Sap, 1, May, 1921. F'ton: 27, iv, 13. Vernon, B.C.: 28, iv, 1924, 2 specimens, E. R. Buckell. Kaslo, B.C.: 10, iv, 1907, Cockle (vial No. 35). Montreal, Que.: 17, v, 1915, J. I. Beaulne. The following are among Tothill's paratypes of *aldrichi* in the Canadian National Collection.

Ottawa: 15, April, 1915 (Genitalia slide No. 12); 1905, James Fletcher; 22, iv, 1906, 2 specimens,—1, 4, 1906, W. Metcalfe; 18, iv, 1915, A. E. Kellet; and one labelled 0.15.4. 1889. O. Ottawa. Jordan, Ont.: 20, iv, 1919, C. H. Curran. One not labelled. Mussellshell, Mont.: 5, 12, 1917, Mont. Exp. Sta. Mac. Coll.: 11/4/1915. Toronto, Can.: 16, iv, 1895, E. M. Walker; April 21, 1897? Hull, Que.: 17, April; 15 ?, 2 specimens. Logan Ut. Jn. 1915. H. R. Hogan.

6. *G. longiforceps* Tothill*

1924 *Gonia longiforceps* Tothill (18) (= *Gonia brevipulvilli* Tothill). (Figs. 6, 6a)

The figures show Tothill's description of the forceps in this species very accurately. "Forceps long and slender being about five times as long as the median width. In dorsal view the sides taper abruptly at the base and then continue almost parallel to the apex. Viewed in profile the dorsal declivity starts well beyond the middle and the ventral edge is arcuate."

* NOTE: Names given by Tothill and ending in the word "—forceps" were published in this form. The type labels, however, carry the Latin genitive form "—forcipis". The names stand as published and the same form has been adopted for new species names involving this word.

Where the word "forceps" itself occurs, apart from names (in the text and key) it has been used in the anglicized form and considered as plural, comparable to the word "scissors". The singular and plural Latin forms "forceps" and "forcipes" seem unnecessarily awkward.

There appears to be no difference between the type and paratypes of this species and those of *Gonia brevipulvilli* Tothill, which is here made a synonym.

Among the paratypes of this species and other material previously grouped here occur specimens that have been segregated to form the new species, *G. discalis*. This species differs from *G. longiforceps* in having the dorsal vestiture of the abdomen long, the setae often attaining a length equal to one-half the length of the segments, and borne almost erect; whereas the dorsal abdominal vestiture of *longiforceps* is short and semi-recumbent.

Gonia longiforceps is recorded as bred from *Agrotis orthogonia* (Noctuidae: Lepidoptera).

Type locality: Lethbridge, Alberta.

Distribution: across Canada and the United States.

Type: No. 784 Canadian National Collection.

7. *G. discalis* new species (Figs. 7, 7a)

Male, length 9 to 13 mm. Dark species. Face and front light yellow, with a silvery sheen in some lights; the front from above has a waxy appearance on the sides; parafacials only slightly narrower at the oral margin than at the vibrissal bases, narrowest width greater than the greatest eye width, with a double or triple row of black setae along the inner edge of the eye, others scattered or in broken rows inside these and the usual short row of heavier bristles along the lower edge of the frontal suture, these bristles graduated in length from the shortest at the top of the row to the longest outside and considerably above the oral vibrissae; genae about half the eye height with a vestiture of slender black setae; antennae blackish often with a greyish sheen in some lights; second antennal segment yellowish with short, heavy, dorsal setae; third segment five to seven times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum and small area preceding it yellow; some yellow on humeri. Legs black. Pulvilli and unguis distinctly shorter than last tarsomere. Wings slightly infuscated basally on the dorsal edge.

Abdomen shining black, segments 3, 4, and 5 pollinose basally, red coloration occurs on segments 3 and 4 and sometimes 5 in varying amounts. The dorsal abdominal vestiture on segments 3 and 4 is long and erect or almost erect. The setae reach a length equal to half the length of these segments.

Forceps similar to those of *longiforceps*, dorsally, wide at the base, tapering rapidly then extending with almost parallel sides to the apex. In lateral view the basal dorsal convexity extends less than one-half the length of the forceps; the apex is expanded dorsally, knob-like.

Type:—Nicola, B.C.: 28, iv, 1922, P. N. Vroom, No. 5003 Canadian National Collection.

Paratypes:—Nicola, B.C.: 23, v, 1922, (vial No. 114),—29, v, 1922, P. N. Vroom. Vernon, B.C.: 13, 4, 1927,—20, iv, 1927, I. J. Ward. Agassiz, B.C.: 29, iii, 1924,—6, iv, 1922. R. Glendenning. Cranbrook, B.C.: 11, v, 1922, 3 specimens,—19, v, 1922,—10, v, 1922,—12, v, 1922,—8, v, 1922, C. B. D. Garrett. Penticton, B.C.: 12, iv, 1927, E. R. Buckell. Copper Mtn., B.C.: 8, iv, 1928. G. Stace Smith. Toronto, Ont.: 13, 4, 1905. Ottawa, Ont.: 22, iv, 1906, W. Metcalfe (Slide No. 13). Earl Grey, Sask.: 25, iv, 1926, J. D. Ritchie. Saskatoon, Sask.: 27, iv, 1923, K. M. King. Lethbridge, Alta.: April 30, 1915, E. H. Strickland.

The following paratypes of *longiforceps* Tothill are in the Canadian National Collection.

Vancouver, B.C.: 10, viii, 1907,—1, 4, 1916, R. S. Sherman. Treesbank, Man.: 17, iv, 1908, J. B. Wallis. Aweme, Man.: 16, v, 1921, N. Criddle. Chilicotin, B.C.: 24, iv, 1920, E. R. Buckell (Genitalia vial No. 115).

aldrichi group

Gonia aldrichi, *G. grandipulvilli*, *G. longiforceps*, and *G. discalis* form a second group of species all closely related. The dark coloration and arcuate ventral edge and apical swelling of the forceps suggest a close relationship to the first or *frontosa* group. In general the forceps in this group are longer and stronger than in the first and the flies are larger. Specimens have been captured throughout the breadth of the United States and Canada during the spring months.

The third or *breviforceps* group, which follows, is also taken in the spring, but the ventral edge of the forceps is straight and the distribution of the species is largely limited to central and western Canada and the United States.

8. *G. breviporceps* Tothill

1924 *Gonia breviporceps* Tothill, (18). (Figs. 8, 8a)

From this species as described by Tothill the following new species, *G. albagenae*, has been separated. The new species includes one paratype of *breviforceps* Tothill, Vernon, B.C., 25, v, 1919. W. B. Anderson, genitalia slide No. 14, Canadian National Collection. *G. breviporceps* has distinctly dark setae on the genae while *G. albagenae* has a vestiture of fine white setae on the genae except for a few darker hairs at the lower edge in some specimens. In general the parafacials are slightly narrower in *breviforceps* Tothill.

G. breviporceps has been bred from *Euxoa ochrogaster* both in Alberta and Saskatchewan.

Type locality: Lethbridge, Alberta.

Distribution: British Columbia, Montana, California, Colorado.

Type: No. 788. Canadian National Collection.

9. *G. albagenae* new species (Figs. 9, 9a)

Male: length 9.5 to 11.5 mm. Specimens usually show considerable red, but dark ones occur. Face and front light yellow, with a silvery sheen in some lights; front from above has a waxy appearance on the sides; parafacials narrowing very little from the arisal bases to the vibrissae, at the narrowest point about equal in width to the greatest eye width, with an uneven double or triple row of slender black setae along the inner edge of the eye and others scattered or in broken rows inside these, the usual short row of graduated bristles along the frontal suture; genae about half eye height, covered with sparse fine white setae, at the most a few dark setae occur next to the basal row of bristles; antennae blackish with a greyish sheen in some lights; second segment yellowish with short heavy dorsal setae, third segment 5 to 7 times as long as second. Pile on the occiput white. Palpi yellow. Dorsum of thorax black with five indistinct longitudinal pollinose lines; scutellum, small lateral areas preceding it, and parts of humeri yellowish. Legs black. Pulvilli and unguis distinctly shorter than last tarsomere. Wings clear with very slight costal infuscation.

Abdomen usually broadly reddish on the sides of segments 3 to 4, the coloured areas sometimes extending to other segments and ventrally, leaving only basal and apical dark rings connected by a narrow dark stripe dorsally or by dark dorsal and ventral stripes. Lobes of fifth sternite dark. Dorsal abdominal vestiture short and semi-erect.

The forceps resemble those of *breviforceps*, short and straight on the ventral edge, little dorsal convexity, dorsally with almost parallel sides for the greater part of their length.

A female (Vaisseaux, B.C., 14, vi, 1919, W. B. Anderson), apparently of the same species has similar body markings and white genal hairs.

Type:—Penticton, B.C.: 12, iv, 1927, E. R. Buckell, No. 5034 Canadian National Collection.

Paratypes:—Penticton, B.C.: 21, iv, 1927, E. R. Buckell (Genitalia vial No. 113). Lillooet, B.C.: ? A. W. A. McPhair. Naramata, B.C.: 2, v, 1919, E. R. Buckell (Genitalia vial No. 132). A paratype of *breviforceps* Tothill in the Canadian National Collection. Vernon, B.C.: 25, v, 1919. W. B. Anderson (Genitalia slide No. 14).

10. *G. setigera* Tothill

1924 *Gonia setigera* Tothill, (18). (Figs. 10, 10a)

Drawings were prepared from the one male paratype (No. 781 Canadian National Collection) with same data as the type. Two male specimens, one from Penticton, B.C., 21, iv, 1927, E. R. Buckell, with forceps exactly as in the paratype and a second from Oliver, B.C., 23, iv, 1927, (Genitalia vial No. 131), E. R. Buckell, of which the forceps show a more evident dorsal convexity but are otherwise similar, are in the Canadian National Collection. Males and females of this species are easily distinguishable by the numerous

setae on the first vein (R_1). The third vein (R_{4+5}) usually bears a greater number of setae than in any other species. As was pointed out in dealing with the generic limits of *Gonia* the generic keys of Williston and Coquillet exclude this species. In examining other species one specimen was noticed with one seta dorsally on the first vein of one wing only.

Type locality: Essex, Mass.

Distribution: Massachusetts, California, British Columbia.

Type: in the Museum of the Boston Society of Natural History.

11. *G. turgida* Coquillet

1897 *Gonia turgida* Coquillet, (3). 1924 *Gonia turgida* Coquillet, (18). 1936 *Cystogonia turgida* (Coq.) Townsend, (21). (Type of new genus *Cystogonia* Townsend). (Figs. 11, 11a).

Drawings were made from one of several male specimens from Idaho, May 2, 1919, E. H. Quales, in the Canadian National Collection, probably placed in this species by Tothill who redescribed the species from a male taken by E. G. Holt at Round Mountain, Nevada, 6,300 ft. (The writer was unable to locate this specimen.) Tothill had his specimen compared with the type by Aldrich, who mentions the striking colour pattern of the type. Among the material received from Saskatoon are two specimens, one from Three Mile Creek, Sask., 23, v, 1921, A. E. Cameron, and one labelled Mortlach, 31, v, 1909, which are evidently the same species. The original description of Coquillet, "the front near the eyes densely covered with rather long bristly hairs, sides of face each one and one-half times as wide as the median depression, densely covered with rather long black bristly hairs which are less numerous along the facial ridge," is sufficient to assure the identity of these specimens. The width of the parafacials compared to the eye width as used in Tothill's key is, alone, however, totally inadequate as a determining character.

Type locality: Los Angeles, California.

Distribution: California, Idaho, Saskatchewan.

Type: No. 3640 United States National Museum.

breviforceps group

The writer considers the straight ventral edge and general shape of the forceps in this group to be indicative of close relationship. The body coloration is characterized by a distinct dorsal, longitudinal dark stripe on the abdomen. Frequently a ventral dark stripe is also present.

There appears to be a decided break between the three groups of species described above; i.e., the *frontosa*, *aldrichi*, and *breviforceps* groups and the two groups to follow. Adults of all species dealt with up to this point are collected in the spring months, while all those to follow are found in the late summer. This suggests a different host relationship and means of spending

the winter. No host records of the following species are known to the writer. Moreover, much wider variations in genital and other characters occur among the following forms. They are, as far as we have evidence, to date, generally more restricted in their habitats as well as specialized in genital structures.

12. *G. sequax* Williston

1887 *Gonia sequax* Williston (24). 1897 *Gonia capitata* (DeGeer). Coquillett (3). = *Gonia frontosa* Say, = *Gonia philadelphica* Macquart, = *Gonia albifrons* Walker, = *Gonia exul* Williston (probably incorrect). 1905 *Gonia capitata* (DeGeer) (1) (follows Coquillett). 1924 *Gonia sequax* Williston (18) (redescribed). (Figs. 12, 12a).

The drawings were prepared from a male specimen from Jordan, Ont., 23, viii, 1915, C. H. Curran, in the Canadian National Collection. This locality is mentioned by Tothill, who no doubt examined this specimen. Four male and five female specimens were taken by the writer and his associates at Macdonald College during August and September, 1938. It is not clear whether Tothill examined the type material of *sequax*, but the large amount of yellow on the body stressed by Williston in his original description together with the short pulvilli make this determination fairly certain. Females taken at Macdonald College show a more widespread darkening on the dorsal surface of the abdomen than do the males which have a very narrow continuous or broken dorsal, dark stripe widening out at the base and apex of the abdomen. No ventral longitudinal dark stripe is present. The genal hairs are yellow.

Synonymy with *G. frontosa* Say can be discarded on colour, size, colour of genal hairs, and the appearance of the forceps. Synonymy with *G. capitata* (DeGeer) is similarly denied by the light colour and the yellow genal hairs. The forceps show a somewhat weaker dorsal convexity than do those of the specimen determined as *G. capitata* (DeGeer) by Bezzi (See Figs. 22, 22a) but are otherwise similar.

Tothill makes no mention of *G. exul* Williston. Coquillett and Aldrich synonymized this species with *sequax* Williston. It seems probable that the female described as *exul* was the female of *sequax*. It is not possible to be certain of this since females cannot be separated as definitely as males. However, this description fits females taken at Macdonald College along with males of *sequax*. The California males with long pulvilli mentioned by Williston and considered conspecific with the described females were most probably *G. longipulvilli* Tothill, while the North Park specimen with narrow parafacials was *G. distincta* Smith.

Type locality: California.

Distribution: California, Colorado, Texas, Connecticut, Massachusetts, British Columbia, Ontario.

Type: ?

13. *G. senilis* Williston

1887 *Gonia senilis* Williston (24). 1897 *Gonia senilis* Williston, Coquillett (3), = *Gonia sagax* Townsend (synonymy probably incorrect). 1905 *Gonia senilis* Williston (1) (follows Coquillett). 1924 *Gonia senilis* Williston, Tothill, (18), (redescribed as distinct from *sagax* Townsend). (Figs. 13, 13a).

Drawings were prepared from a specimen in the Canadian National Collection labelled, Oak Grove, Virginia, Fla., Dacus, 2, viii, ?, C. H. Townsend. This locality is mentioned by Tothill in his redescription of this species. Among other specimens examined were: one male from Severn, Ont., 16, 6, 1925, C. H. Curran; one male from College Park, Indiana, W. R. Walton; and two females apparently of the same species from Robinson, Delaware Co., Iowa, vii, 24, 1924, N. K. Bigelow. The suggested synonymy of this species with *sagax* Townsend is incorrect if the specimens available are accurately determined. The differences in the forceps, which are shorter and much broader in *senilis* Williston, are evident from the diagrams. Tothill separated the two species on the presence of dark setae on the genae in *sagax* and yellow setae in *senilis*. In the male from Severn, Ont., and one female from Delaware, at least numerous of the basal genal hairs are dark in some lights. The colour of the arista, which in the original descriptions is given as orange yellow except at the tip in *senilis* and as brown in *sagax*, appears to be a good character but should be borne out by the forceps.

The differences in the forceps together with the fact that *sagax* has been captured in the spring months and *senilis* in the summer has led the writer to adopt the grouping in this paper and separate these two species so widely in spite of the similar colour of the third antennal segments.

Type locality: Western Kansas.

Distribution: Indiana, Virginia, New Jersey, Georgia, Florida, Ontario.

Type: ?

14. *G. distincta* Smith

1915 *Gonia distincta* Smith (15). 1916 *Gonia distincta* Smith, Townsend (20), (made the type of a new genus *Cnephalogonia* Townsend). 1924 *Gonia distincta* Smith, Tothill (18). (Figs. 14, 14a, 14b).

Gonia distincta Smith was described from three female specimens and the original description, except for the colour, gives us little information of specific value, especially as the specimens were female. Townsend, making this the type of his new genus, adds: "Female. Front not swollen . . . no median marginal macrochaetae on the first* abdominal segment; no closely set marginal macrochaetae on the third* segment. Parafacials below not over one-half greatest eye width, widening above to nearly eye width at base of antennae. Front marginal macrochaetae of parafacials sparse, few and weak."

* "First" and "third" refer to the "second" and "fourth" segments respectively, as understood in the second part of this paper.

Tothill redescribed the species from five males and two females, one of the females having been compared with the type described by Mr. C. W. Johnson. The narrow parafacials are mentioned and the "abdomen in males yellow with a wide black dorsal stripe that spreads out posteriorly to cover part of the third and all of the fourth tergum, and with a median ventral longitudinal black stripe also; in the females, black." The forceps are also briefly described.

Townsend (21), in his Manual of Myiology, separates his genus *Cnephalogonia*, of which this species is the type, from related genera on the absence of median marginals on the first segment. This character is purely sexual, these median marginals being present in males of this species and absent in the females of many other species.

The writer prepared his diagrams from a specimen labelled Bar Harbor, Me., 3, viii, 24 C. W. Johnson in the Canadian National Collection, one of the specimens studied by Tothill. Other specimens examined included: two males from Mt. Desert, Me.; two from Aweme, Man.; two from Blackburn, Ont.; 11 males from Low Bush, Ont.; eight females from Low Bush; two from Blackburn and one from Mt. Desert, all in the Canadian National Collection, and one male from Bozeman, Montana.

It seems of value to redescribe this species more fully or at least fill in previous descriptions.

Male: length, 9 to 12 mm. Face and front much less swollen than in any other species of *Gonia*. Parafacials much narrower at the vibrissae than at the arisal bases with two or three uneven rows of setae, which increase in size mesally, and the usual row along the frontal suture. No bare space between other setae and this last row. Genal setae dark, genae about one-third eye height. Occipital pile white. The dorsum of the thorax in certain lights appears to have the usual five wide longitudinal, pollinose lines separated by narrow dull dark lines. In other lights the narrow dull black lines appear pollinose, the wide lines shiny black. The scutellum, varying areas cephalic to the scutellum, and the humeri, yellow. Legs black. *Pulvilli and ungues longer than the last tarsal segment.* Pulvilli white. Ungues slender, curved at the tips and in strong light yellow except at the tips. The abdomen has been well described as to colour by Tothill (quoted above). In most cases it is more orange than yellow. The dorsal and ventral longitudinal stripes are constant and conspicuous; the segmental bases are broadly pollinose. The pollen spreads out especially on the sides of segments 4 to 5 almost the length of the segments. A pair of marginal macrochaetae are present on the second (Townsend's first) segment and a second pair on the third segment. The fourth bears a row of long, strong, marginals, the fifth a row of sub-marginals with a row of shorter bristles caudad of these.

The forceps are covered on the dorsal side with long, slender, black setae; the dorsal convexity extends down almost to the apex giving them a singular resemblance to those of *longipulvilli* Tothill; however, the extension of the convexity in the last mentioned species is not as great.

Female. Face less swollen than in male, appearing almost flattened. Parafacials wider than in male, considerably narrower at vibrissae than at arisal bases, width at vibrissae about two-thirds greatest eye width.

Thorax as in male. Pulvilli and unguis variable in individuals, from two-thirds to full length of last tarsomere; unguis slender and yellowish basally. Abdomen shining black with only obscured reddening on the sides and venter, no median marginals on the second segment.

Segments pollinose basally, pollen spreading laterally on segments 4 and 5.

Type locality: Westport Factory, Mass.

Distribution: Mass., Conn., Maine, Man., Sask., Mon., Ont.

Type: collection of Boston Society of Natural History.

The North Park specimen referred to by Williston when describing *Gonia exul* was probably of this species.

15. *G. longipulvilli* Tothill

Gonia longipulvilli Tothill (18). (Figs. 15, 15a, 15b)

The drawings were prepared from type No. 789 in the Canadian National Collection. Specimens examined have the pulvilli and unguis of the male longer than the last tarsomere; a narrow or broken dark, longitudinal dorsal line on the abdomen, the remainder of the abdomen laterally and ventrally except for the very base and the apex suffused with an orange yellow colour, no ventral longitudinal dark line being present; forceps with the dorsal convexity extending about three-quarters of their length. The California specimens mentioned by Williston in section (a) following his description of *exul* were probably of this species. This species has been bred from *Agrotis orthogonia* in Saskatchewan.

Type locality: Royal Oak, B.C.

Distribution: the central and western parts of North America from north to south.

Type: in the Canadian National Collection.

16. *G. porca* Will.

1887. *Gonia porca* Williston (24). 1924 *Gonia porca* Williston, Tothill (18), (redescribed). (Figs. 16, 16a)

Drawings were made from a specimen labelled Lillooet, B.C. Aug., 1917, one of the specimens examined by Tothill during his study. As Tothill has pointed out, the yellow hair on the pleurae and the mesonotum make identification of this species simple as do the yellow lobes of the fifth sternite and the shape of the forceps. The depth and chisel-like apex of the forceps is an extreme modification which sets this species very distinctly apart.

Several previously undetermined specimens are in the Canadian National Collection from Jesmond, B.C., 23, vii, 1932, J. K. Jacob, at altitude 7,000

to 7,500 ft., and a series from Lake Louise, Banff, Alta., 22, vii, 1938, G. S. Walley, 8,600 ft.

Type locality: Mt. Hood, Oregon.

Distribution: Oregon, British Columbia, Alberta, Colorado, North Mexico. (Mountainous regions).

Type: ?

17. *G. texensis* Reinhard

1924 *Gonia texensis* Reinhard (12). (Figs. 17, 17a)

The drawings were prepared from paratype No. 791 in the Canadian National Collection, with the same data as the type. This species has been adequately described including the forceps. These latter structures resemble those of *G. porca* Williston in their unusual depth but are greatly modified in shape and vestiture setting this species, too, distinctly apart from all others.

Reinhard says, "In relationship this species is probably nearest to *angusta* Macquart which in Aldrich's catalogue is listed as a synonym of *pallens* Wiedmann, described from Brazil."

(*Gonia angusta* Macquart was described in *Diptera Exotique*, Paris, Vol. II (3): 51. The author notes that the abdomen is narrower than the thorax, a condition peculiar to *texensis* in our fauna. In 1849 Walker, in *Diptera* in the British Museum iv: 797, records *angusta* Macq. from Jamaica.)

Type locality: College Station, Texas.

Distribution: Texas.

Type: United States National Museum, Washington.

sequax group

The above six species form the *sequax* group. The forceps are, in general, deep. Like the group to follow, and unlike the preceding three groups, they are collected in the late summer. The last two species, at least, are widely divergent from the close relationships so evident among other species and are similarly of limited habitat. It seems probable that transition forms between these and other types may exist.

The last or *fissiforceps* group consists of three species so closely related as to cause some doubt as to their specific rank and yet so widely separated from all the other species as to stand alone. Their habitat only they have in common with *porca* Williston, and their occurrence in late summer in common with the *sequax* group.

18. *G. fissiforceps* Tothill

1924 *Gonia fissiforceps* Tothill (18). (Figs. 18, 18a, 18b)

This large, dark, mountain species is easily recognizable from the diagrams given here and from the description by Tothill. It might be added that the

palpi vary from yellow to black, the pulvilli are white and almost as long as the last tarsomere in males. The "escaloped" inner edges of the lobes of the fifth sternite are characteristic of this and the following two species.

The new species *tenuiforceps* is being separated from this species on the shape of the forceps alone. One of Tothill's paratypes labelled Banff, Alta., N. B. Sanson, belongs to the new species.

Type locality: Lillooet, B.C.

Distribution: Banff (Alberta), Colorado, Washington, California, Ontario.

Type: No. 782, Canadian National Collection.

19. *G. tenuiforceps* new species. (Fig. 19, 19a)

Male: length, 10 to 11 mm. Shiny black species. Face and front light yellow, with a silvery sheen in some lights; the front above with a waxy appearance on the sides; parafacials not narrowing greatly from opposite arisal bases to oral margin, at the narrowest point slightly less than or equal to the greatest eye width, with a double or triple irregular row of slender black setae along the inner edge of the eye. The usual row of setae along the edge of the frontal suture irregular in extent and in size of bristles; genae about one-half the eye height with a few scattered black setae. Antennae black with a greyish tinge in some lights, second segment reddish or yellowish apically with short, dark, dorsal setae; third segment five to seven times as long as the second. Pile on the occiput white. Palpi yellow through various degrees of infuscation to black. Thorax shiny black, sometimes with powdering of white pollen, which may be distinctly divided into five lines by very narrow shiny areas; scutellum yellow; legs black, femora and tibiae (each or both) in strong light may show yellow areas. Pulvilli and unguis (about) as long as last tarsomere. Pulvilli white. Unguis slender, and, in strong light, yellow at the base and tip.

Abdomen shining black, segments 3, 4, and 5, sometimes narrowly, evenly white pollinose basally. Rarely with indications of yellowish red colouring on the sides of segments 2, 3, and 4. The lobes of the fifth sternite are definitely excised laterally on the inside, giving a "scalloped" effect (Fig. 18b), as in *fissiforceps* and *yukonensis*.

Forceps long, resembling those of *fissiforceps* but having much less depth medially, dorsal convexity extending less than one-third of their length, apex turned ventrally (forward) to form a hook, in dorsal view the median apical cleft extends beyond the middle.

Type: Male, Hopedale, Labrador, 21, vii, 1926, W. W. Perret,—No. 5035 Canadian National Collection.

Paratypes: As above; 23, vii, 1926,—17, vii, 1926,—25, vii, 1924,—10, vii, 1926,—22, vii, 1926. Two females 18, vii, 1926. One paratype of *fissiforceps* Tothill, Banff, Alta., ?, N. B. Sanson.

20. *G. yukonensis* Tothill

Gonia yukonensis Tothill (18). (Figs. 20, 20a, 20b)

Drawings were prepared from the type, No. 786, Canadian National Collection.

Tothill records a paratype of this species from Tennessee Pass, Colo. (in the United States National Museum). His description of the median apical cleft of the forceps "extending about one third distance to base" proved a little inaccurate, when the forceps had been treated in potash. The cleft is seen to extend almost half the distance to the base. The similar habitat and the shape of lobes of the fifth sternite (not mentioned by Tothill) suggest a very close relationship with *fissiforceps*. The forceps, however, have the dorsal convexity extending further down the length of these structures (about one-third the distance to the apex) than in any *fissiforceps* observed. For the present it is thought best to maintain this name but there is considerable doubt in the writer's mind as to the specific rank of this specimen.

Type locality: Yukon Territory.

Distribution: Yukon, Colorado.

Type: No. 786, Canadian National Collection.

21. *G. carinata* Tothill

1924. *Gonia carinata* Tothill (18).

There being no known specimens of this species besides the holotype, which was not available to the writer, diagrams of this species could not be included. The following is Tothill's original description:

"Parafacials at narrowest point about three times the length of the second antennal segment and only slightly wider at the base of the antennae than at vibrissae. Genal hairs fuscous. Third antennal segment black. Occipital pile white. Pleura without golden pile. First vein without bristles; third vein with a few small bristles at base. Abdomen reddish yellow except for a dorsal and a ventral narrow black stripe and except for the last tergum which is black.

"The forceps of genitalia remarkable for the long median carina that is as deep as the forceps are thick. Otherwise the forceps are long and narrow with the notch confined to the apical fourth, and straight in ventral profile."

Holotype: Male; from Salt Lake, Utah; in United States National Museum.

Tothill merely listed the species in the order in which they occurred in his key, though he suggested a western origin and eastward spread of this genus. The information at hand seems inadequate to support or deny this hypothesis. However, the tentative order of the species in this paper is based on distribution, time of occurrence, and especially similarities in the structure of the forceps.

Frontosa is our most widely spread species, *fuscicollis* is undoubtedly closely related to it, and *sagax* though unique in its antennal colour resembles these two in the structure of the forceps. In the *aldrichi* group the forceps maintain to some extent their curved ventral edge and dorsal apical convexity but have become elongated and flattened. In the *braviforceps* group the ventral edge of the forceps has become straight and an increase in depth is evident. Moreover, the range appears more restricted. The *sequax* group includes species from widely separated areas. They are separated from each other more distinctly than are the members of other groups. In all these species the forceps have increased markedly in depth. The *fissiforceps* group includes, as previously noted, extremely closely related species, but as a group shows no close affinities to any other species. The habitat of the last group is limited to mountainous areas. It may thus overlap the habitats of *porca* and possibly *distincta*. The shape of the lobes of the fifth sternite and the shape of the forceps set the *fissiforceps* group apart.

Since *carinata* could not be examined nor even the date of capture ascertained it could not be fitted into this arrangement and is consequently listed last.

Acknowledgments

The gratitude of the writer is due to many people who have assisted in making this work possible. Among those to whom especial thanks are due are: Prof. E. H. Strickland, Professor of Entomology, University of Alberta, for suggesting the problem and putting his collection at the writer's disposal; Mr. K. M. King, Entomologist in Charge, Science Service, Entomological Laboratory, Saskatoon, Saskatchewan, for the loan of specimens and for data on rearing; Dr. Harlow B. Mills, Assistant State Entomologist, Boseman, Montana, for the loan of specimens; Dr. D. J. McDunnough, Chief, Systematics, Division of Entomology, Department of Agriculture, Ottawa, for graciously allowing the writer to spend weeks, on two occasions, examining type and other material in the Canadian National Collection; Dr. McDunnough's staff for their kind co-operation and most valuable advice; Dr. E. M. DuPorte, Macdonald College, for valuable suggestions and assistance; Dr. W. H. Brittain and Dr. E. M. DuPorte for the use of the facilities of Macdonald College; Mr. W. E. Whitehead, Macdonald College, for advice and liberal assistance in preparing, lettering, and photographing the figures; and Dr. J. W. McB. Cameron for suggestions in arranging and correcting the manuscript. To these and many others the writer is profoundly grateful for help and encouragement.

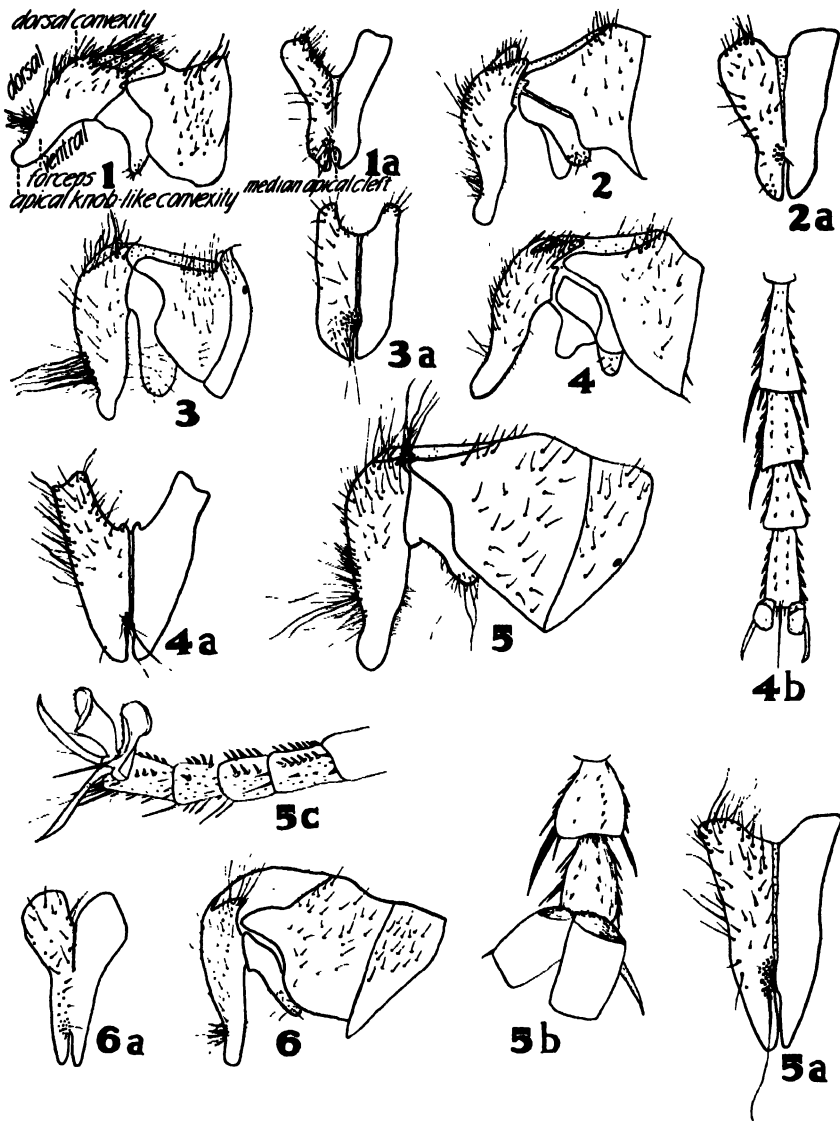
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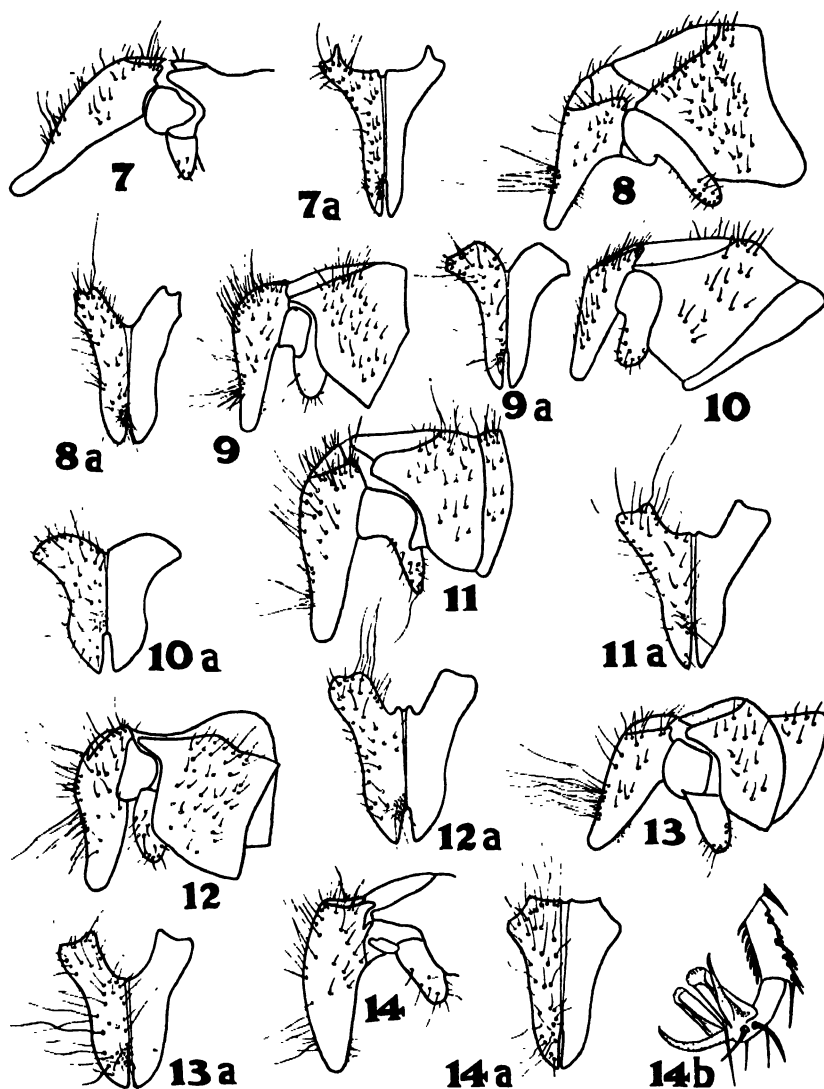
Explanation of Figures

Figs. 1 to 22 have been prepared in so far as possible from type material. In many cases the sclerites behind the forceps were broken or twisted and were drawn as observed. The exact position of the structures when drawn was that in which the forceps were most distinctive, and it varies slightly with different specimens. In all cases it is the contour of the forceps and not the exact shape of the other sclerites that is specific. The density, distribution, and length of setal vestiture varies and is often affected by the way material has been handled. It has, however, been indicated so far as possible. The linear magnification is in all cases approximately 30.



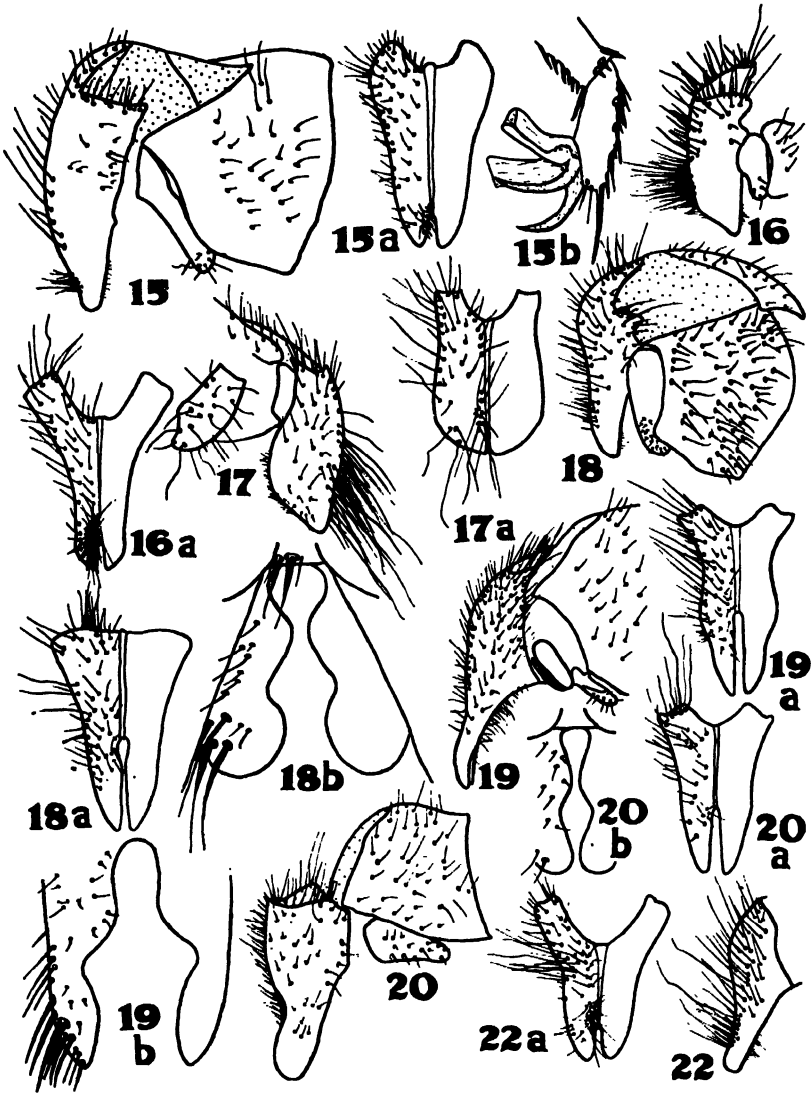
Figs. 1 to 6a.

1. Lateral view of the forceps of *G. frontosa* Say. 1a. Dorsal view of the forceps of *G. frontosa* Say. 2. Lateral view of the forceps of *G. sagax* Townsend. 2a. Dorsal view of the forceps of *G. sagax* Townsend. 3. Lateral view of the forceps of *G. fuscicollis* Tothill. 3a. Dorsal view of the forceps of *G. fuscicollis* Tothill. 4. Lateral view of the forceps of *G. aldrichi* Tothill. 4a. Dorsal view of the forceps of *G. aldrichi* Tothill. 4b. Anterior tarsus, claws and unguis of *G. aldrichi* Tothill. 5. Lateral view of forceps of *G. grandipulvilli* new species. 5a. Dorsal view of the forceps of *G. grandipulvilli* new species. 5b. Dorsal view of last two tarsomeres, pulvilli and unguis of the prothoracic leg of *G. grandipulvilli*, new species. 5c. Lateral view of the same. 6. Lateral view of the forceps of *G. longiforceps* Tothill. 6a. Dorsal view of the forceps of *G. longiforceps* Tothill.



Figs. 7 to 14b.

7. Lateral view of the forceps of *G. discalis* new species. 7a. Dorsal view of the forceps of *G. discalis* new species. 8. Lateral view of the forceps of *G. breviforceps* Tothill. 8a. Dorsal view of the forceps of *G. breviforceps* Tothill. 9. Lateral view of the forceps of *G. albagenae* new species. 9a. Dorsal view of the forceps of *G. albagenae* new species. 10. Lateral view of the forceps of *G. setigera* Tothill. 10a. Dorsal view of the forceps of *G. setigera* Tothill. 11. Lateral view of the forceps of *G. turgida* Coquillett. 11a. Dorsal view of the forceps of *G. turgida* Coquillett. 12. Lateral view of the forceps of *G. sequax* Williston. 12a. Dorsal view of the forceps of *G. sequax* Williston. 13. Lateral view of the forceps of *G. senilis* Williston. 13a. Dorsal view of the forceps of *G. senilis* Williston. 14. Lateral view of the forceps of *G. distincta* Smith. 14a. Dorsal view of the forceps of *G. distincta* Smith. 14b. Last tarsomere, pulvilli and unguis of the prothoracic leg of *G. distincta* Smith.



Figs. 15 to 22a.

15. Lateral view of forceps of *G. longipulvilli* Tothill (sclerites of segment 9 badly twisted). 15a. Dorsal view of forceps of *G. longipulvilli* Tothill. 15b. Last tarsomere, pulvilli and ungues of the prothoracic leg of *G. longipulvilli* Tothill. 16. Lateral view of the forceps of *G. porca* Williston. 16a. Dorsal view of the forceps of *G. porca* Williston. 17. Lateral view of the forceps of *G. texensis* Reinhardt (sclerites of segment 9 badly broken). 17a. Dorsal view of the forceps of *G. texensis* Reinhardt. 18. Lateral view of the forceps of *G. fissiforceps* Tothill (sclerites of segment 9 badly broken). 18a. Dorsal view of the forceps of *G. fissiforceps* Tothill. 18b. Lobes of the fifth sternite of *G. fissiforceps* Tothill. 19. Lateral view of the forceps of *G. tenuisforceps* new species. 19a. Dorsal view of the forceps of *G. tenuisforceps* new species. 19b. Lobes of the fifth sternite of *G. tenuisforceps* new species. 20. Lateral view of the forceps of *G. yukonensis* Tothill. 20a. Dorsal view of the forceps of *G. yukonensis* Tothill. 20b. Lobes of the fifth sternite of *G. yukonensis* Tothill. 22. Lateral view of the forceps of *G. capitata* (DeGeer)? 22a. Dorsal view of the forceps of *G. capitata* (DeGeer)?

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

OCTOBER, 1940

NUMBER 10

FROZEN STORAGE OF POULTRY

IV. FURTHER OBSERVATIONS ON SURFACE DRYING AND PEROXIDE OXYGEN FORMATION¹

By W. H. COOK² AND W. H. WHITE²

Abstract

A package constructed from moisture resistant material, capable of being ventilated during chilled storage, and sealed to prevent surface drying during frozen storage, is described. Results are presented to demonstrate the ability of this package to maintain the desired humidity conditions. Jacketing a room to separate the cooling coils from the space occupied by the product does not prevent surface drying of boxed goods, presumably because of the absorption of moisture by the boxes. Delays between slaughter and freezing accelerate the development of rancidity in the fat of poultry during subsequent frozen storage, as indicated by the formation of peroxide oxygen. The free fatty acid content is not seriously affected unless the conditions prior to freezing enhance microbial development.

Introduction

It has been shown in earlier papers of this series (1, 2, 3) that surface drying, causing a loss of bloom and development of freezer burn, was the first type of deterioration to occur in poultry during frozen storage. It was also found that conditions favouring surface drying also promoted the development of rancidity in the fat. These results suggested the present studies on methods of packaging for minimizing surface drying, and the effect of delays between slaughter and freezing on the development of rancidity.

The results of previous investigations showed that surface drying could be minimized by lining the boxes with reasonably moisture-resistant stocks, such as waxed paper, provided the folds and joints were adequately sealed. Since adequate sealing of the liners used in wooden poultry boxes is commercially impracticable, other types of packages were studied. One disadvantage of a sealed package is that it maintains a high humidity within the package under all conditions, and during chilled (unfrozen) storage such a condition enhances microbial growth. As a certain proportion of market poultry is held in the chilled state for immediate domestic consumption there is an obvious need for a package that can be readily ventilated or sealed in accordance with trade requirements.

¹ Manuscript received July 23, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 51 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 949.

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Packaging Experiments

Description of Packages

Initial experiments were undertaken with several standard types of corrugated cartons. Those having telescoping covers proved to be best from the standpoint of packing, storing, and sealing. Commercial trials indicated that the strength of such a package was generally satisfactory for a six-bird size. Standard 12-bird packages were also used experimentally, and the use of heavy corrugated stocks and containers of suitable design would appear to make this size entirely practicable for net weights up to 50 or 60 lb.

Cartons of this type must be rendered moisture-resistant. This was accomplished by various methods, including the application of wax or aluminium foil on one or both sides of the carton. Although the foil has many desirable features, the results of these preliminary tests indicated that the application of a sufficiently heavy coating of wax to produce a glossy surface on the inside was adequate for protecting the product. For commercial use the application of wax to the outer surface would also seem desirable to protect the package against condensate and other contact with moisture. All subsequent tests were conducted with both inside and outside surfaces waxed. No liners were used.

Although the ordinary type of full-telescoping carton was easier to seal than the liners in wooden boxes, it was still found difficult to obtain an effective seal in routine practice. This led to the design of the half-telescoping carton shown in Fig. 1, which proved to be comparatively easy to seal, and also facilitated storage of the product in either a "ventilated" or sealed condition. In this package the full size inner tray fits into another tray of half the height, and is covered by a similar half-height tray. These two cover members meet along the medial line of the container, and can be effectively sealed with a moisture-resistant adhesive tape. This construction also provides a double bottom to strengthen the package. If necessary, the carton can be strapped or wired before storage or transport.

The necessary ventilation during chilled storage was obtained by providing a hole in each end of the inner tray just above the joint of the two outer members. By suitably stamping the telescoping cover opposite these holes it was possible to provide a flap that could be broken open to expose the holes when ventilation was desired, or sealed beneath the tape to provide a moisture-tight package. Previous tests demonstrated that a relative humidity range of 85 to 90% was obtained during chilled storage when the openings in the inner tray were $\frac{1}{2}$, $\frac{3}{4}$, and 1 in. for boxes designed to contain about 25, 40, and 60 lb. of poultry respectively. Liners were not used in these packages since they were unnecessary and might obstruct the openings.

Results

Semi-commercial scale tests were conducted with the new design of package in both chilled and frozen storage. The relative humidity inside the package was taken as the criterion of proper ventilation in the chilled state, while the

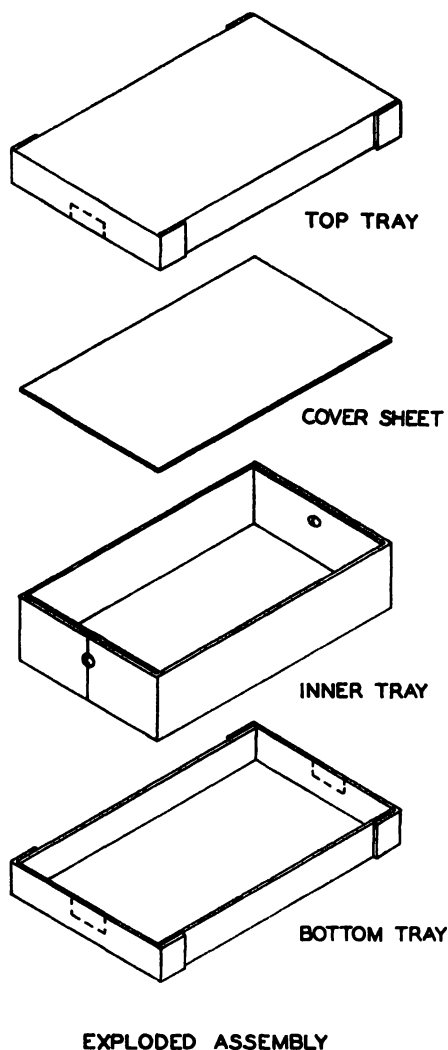


FIG. 1. *N.R.C. design of corrugated carton used to facilitate ventilation or sealing.*

proportion of the surface area affected by freezer burn after 27 to 32 weeks' storage at -12°C . was used as a measure of its value for protecting the frozen product against drying.

The chilled storage tests were of 30 days duration at 0°C . During this period two relative humidity measurements were made on each of the experimental boxes using the special hair hygrometer referred to in earlier work (1). Observations were made on both ventilated and sealed packages of the type described earlier, and also on a few of the older types of containers. The results in Table I show that the ventilated N.R.C. type maintained relative humidities between 85 and 90%, which is considered satisfactory. This package in the sealed form, and all the other containers tested, maintained

TABLE I
RELATIVE HUMIDITY IN POULTRY BOXES DURING 30 DAYS' STORAGE AT 0° C.

Type of box	No. boxes in test	Range of size (net wt. poultry, lb.)	Relative humidity inside boxes during storage		
			Max.	Min.	Av.
N.R.C.—ventilated	5	19-75	90	84	86
N.R.C.—sealed	5	22-74	100	98	99
Ordinary telescoping carton—unsealed	1	28	—	—	98
Wooden box with unsealed moisture-resistant liner	2	42-50	98	100	99

humidities approaching saturation, which is clearly too high for satisfactory storage in the chilled state.

The extent of surface drying in the various containers stored in the frozen state is evident from the results presented in Table II. The average value of 2% indicates that the new containers give adequate protection from surface drying. For the most part the small freezer-burnt areas occurred only on certain birds that had been forced in close contact with lightly waxed regions of the cartons. Experienced inspectors examining these boxes reported no evident deterioration, and classed the bloom as "good" or "excellent".

Although only a limited number of other types of boxes were available for comparison, the results are in complete agreement with those obtained in earlier preliminary experiments. An ordinary waxed but unsealed telescoping

TABLE II
SURFACE DRYING DURING STORAGE IN FROZEN STATE

Type of box	No. boxes in test	Storage conditions	Proportion of surface area affected by drying			Remarks
			Max., %	Min., %	Av., %	
N.R.C.—sealed	8	27 weeks at -12° C. 65% R.H.	8	1	2	Figures exclude one damaged box showing 10% F.B.
N.R.C.—sealed	11	32 weeks at -12° C. 65% R.H.	3	1	2	
Ordinary telescoping carton—unsealed	2	32 weeks at -12° C. 65% R.H.	30	3	14	
Wooden box with unsealed moisture-resistant liner	1	32 weeks at -12° C. 65% R.H.	—	—	14	
Wooden box with sealed moisture-resistant liner	2	27 weeks at -12° C. 65% R.H.	3	1	2	

carton, and the usual wooden box with unsealed liner both showed about 14% of the area of the product affected by freezer burn. Inspectors reported deterioration in these boxes. Sealing the waxed paper liner in the wooden boxes reduced the affected area to 2%, in agreement with earlier results (1).

One of the recent developments for preventing drying and shrinkage of individually wrapped birds during storage is the use of thin transparent latex bags. Ten birds were sealed in these bags and exposed, without further protection, for a period of 43 weeks to the conditions described earlier. At the end of the storage period there was no evidence of drying, and the loss in weight was less than 1%.

Experiments in Jacketed Spaces

Primarily, a moisture-resistant packaging provides a vapour barrier between the product and the cooling coils, the regions of maximum and minimum vapour pressure respectively. The introduction of this vapour barrier in the same relative location, but as part of the room rather than as part of the package, might prove equally effective and less costly in the prevention of drying. The use of a jacketed room, as suggested by Huntsman (4), with the cooling coils placed between the jacket and the insulated wall, appears to meet these requirements. Although a cold store of this type would doubtless reduce desiccation, it might not be as effective as moisture-resistant packaging, since there is some evidence (5) that packages such as wooden boxes may themselves absorb considerable moisture from the air at relative humidities approaching saturation. In these circumstances the cooling coils may not be the only agency responsible for drying, and consequently their isolation from the space occupied by the product may be only partially effective in reducing desiccation.

This possibility was examined by placing two boxes of poultry in each of two gas-tight steel tanks to represent jacketed spaces. These were of sufficient size to contain two boxes of poultry in $\frac{2}{3}$ to $\frac{3}{4}$ of their volume. Approximately 20 lb. of ice was placed in the bottom of one of the tanks, in order to provide a source of water vapour, other than the product, for the maintenance of a high relative humidity. The other tank contained only the boxed product. In all instances the poultry was packed in wooden boxes with moisture-resistant unsealed liners. The tanks were stored at a temperature of from -12 to -15°C . for a period of 87 weeks before being opened for examination. This prolonged storage period was used to exaggerate any surface desiccation that might have occurred.

Results showing the condition of the poultry in the two tanks at the end of the storage period are given in Table III. Serious deterioration of the product had occurred in the tank without ice, while that in the tank containing ice did not show marked injury. Although this product was stored for an excessively long period, the results nevertheless demonstrate that the absorption of moisture by the package may cause serious surface desiccation. In these circumstances it is evident that goods packed in containers capable

TABLE III
SURFACE DRYING FOLLOWING 87 WEEKS' STORAGE AT
-12 TO -15° C. IN GAS-TIGHT TANKS WITH
AND WITHOUT ICE

Storage conditions	Surface area affected by desiccation, %	Bloom
In tank without ice	20-25 10-15	Poor Poor
In tank with ice	0-5 0-5	Good Excellent

of absorbing moisture cannot be stored successfully in a jacketed room unless a high humidity is maintained by some agency other than the stored product.

Formation of Peroxide Oxygen and Free Fatty Acids

The results of a previous investigation on the frozen storage of poultry showed that the fat was relatively resistant to oxidative and hydrolytic changes (3). Although surface desiccation accelerated peroxide oxygen formation, it was found that, even under conditions favouring severe drying, the peroxide oxygen content seldom exceeded 8.0 ml. of 0.002 *N* sodium thiosulphate after a storage period of 25 months at -13.5° C. It was concluded that the extent of the decomposition of the fat should seldom effect serious deterioration in the flavour of poultry which was promptly precooled and stored at suitable temperatures in the frozen state for normal storage periods.

In commercial practice, delays in cooling or freezing may unavoidably occur, and if sufficiently prolonged may result in a considerable acceleration of the decomposition of the fat. Although such changes may not be evident immediately because of the nature of the induction period characteristic of the development of rancidity, the fat may become rancid quite rapidly during a subsequent period of frozen storage. The material available from the packaging experiments described previously permitted some preliminary observations on this problem.

Peroxide oxygen and free fatty acid determinations were made by methods previously described (3), on the subcutaneous and skin fat of one or two birds taken at random from each of a number of the boxes at the end of the period of frozen storage. This material represented poultry that had been precooled and packed in a commercial plant, and which had been held at temperatures of approximately 0° C. for periods of one week and five to six weeks between slaughter and freezing. Since none of the boxes from which the samples were taken suffered evident deterioration from drying, the accelerating action of surface desiccation on peroxide oxygen formation was excluded.

The peroxide oxygen and free fatty acid contents of the fat and the corresponding storage conditions are shown in Table IV. Poultry held for one week at 0° C., followed by frozen storage at -12° C. for 32 weeks, yielded peroxide oxygen values approximately twice as large as those previously obtained for poultry stored for much longer periods (3). This suggests that delays before precooling or freezing were responsible for the greater deterioration. The free fatty acid contents for this group of poultry were low and approximately normal.

TABLE IV

PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF POULTRY FAT FOLLOWING VARIOUS STORAGE TREATMENTS

Storage conditions	Peroxide oxygen (as ml. 0.002 <i>N</i> Na ₂ S ₂ O ₃ per gm.)	Free fatty acid as % oleic acid
Precooled commercially, stored one week at 0° C., and 32 weeks at -12° C.	4.0	0.57
	2.7	0.59
	3.0	0.67
	0.8	0.54
	4.9	0.65
	1.7	0.53
Precooled commercially, stored 5 to 6 weeks at 0° C., and 27 weeks at -12° C.	6.3	5.3
	8.8	19.9
	11.1	15.2
	5.1	31.4
	18.4	12.9
Precooled commercially, frozen and stored in a gas- tight tank containing ice for 87 weeks at -12 to -15° C.	9.3	0.95
	6.3	0.60
	6.4	0.88
	11.7	0.89

The next series of samples studied were obtained for poultry stored for five to six weeks at 0° C., followed by storage for 27 weeks at -12° C. Under these conditions the peroxide oxygen values had increased to levels at which the fat would be considered rancid. In addition the free fatty acid content was high, and would undoubtedly exert a deleterious effect on flavour. The excess free fatty acid formation may be attributable to excessive microbial activity during storage at 0° C. These results show conclusively that the product should be precooled and frozen promptly after slaughter in proper storage practice.

It is evident that there is considerable variation in the peroxide content of the fat of birds treated similarly. This indicates a difference in the susceptibility of the fat of different birds to oxidation, and is in agreement with previous findings (3). Doubtless the breeding and feeding of the poultry affects the susceptibility of the fat to oxidation.

The last section of Table IV gives the results of the analysis of the fat of two birds taken from each of the two boxes stored in the gas-tight tank con-

aining ice (see Table III). The peroxide oxygen values were generally higher than those observed previously (3). This may be due to greater delays in precooling and freezing the present material, or to a greater susceptibility of the fats of these birds to oxidation. Nevertheless it is evident that poultry fat may become slightly or definitely rancid under storage conditions that prevent surface drying. Rancidity development is therefore one of the factors limiting the storage life of poultry stored at -12°C . even when surface drying is prevented. These birds were stored for an excessive period, however, and under commercial storage practice, surface drying, which is directly detrimental to quality, and indirectly accelerates oxidative changes, is likely to be the primary factor limiting the storage life.

Acknowledgments

The authors wish to acknowledge the assistance of Messrs. A. F. Chadderton and E. G. Blake, laboratory assistants, National Research Laboratories, Ottawa.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

NOVEMBER, 1940

NUMBER 11

STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

VIII. COMPARISONS OF THE LETHAL EFFECTS OF SOME CHEMICALS CONTAINING SULPHUR ON THE FREE-LIVING STAGES OF *SCLEROSTOMES*¹

By I. W. PARNELL²

Abstract

The effect on the free-living stages of *sclerostomes* of 14 common chemicals containing sulphur is discussed. Potassium xanthogenate in weak solution will sterilize about 160 times its weight of fresh faeces; in a strong solution, less than a quarter of the weight. Carbon disulphide will sterilize only about 32 times its weight under the conditions imposed by this technique. In solutions of medium strength the sulphates of zinc will sterilize 32 times, and those of iron (ferrous), copper, and manganese, 13 times their weight; lesser quantities, however, might be effective in practice, because the larvae from cultures so treated die comparatively rapidly after reaching the third stage. Dry ferric sulphate will sterilize 20 times its weight. Sodium sulphide, sodium sulphite, and sodium sulphate are all so ineffective that only when applied dry or as strong solutions are they lethal; they sterilized from eight to five times their weight. Magnesium sulphate will sterilize only four times and zinc sulphide twice its weight of fresh faeces. Ferrous sulphide and flowers of sulphur do not appear to have any lethal value, but the latter may, by controlling antagonistic factors, increase the chances of survival of the larvae.

Introduction

This paper reports on the lethal properties, against the free-living stages of *Sclerostomes* in fresh faeces, of some chemicals containing sulphur.

Potassium xanthogenate and carbon disulphide were tested because they have, under some conditions, proved effective against plant nematodes. Cupric sulphate was tested because it has for many years been suggested for treating pastures to kill nematode eggs and larvae, is used on pastures to kill snails, and is the standard chemical to use as an anthelmintic against some adult bursate nematodes. Flowers of sulphur was tested because it is the standard lethal agent against certain pests. Zinc sulphate, ferric sulphate, ferrous sulphate, manganous sulphate, sodium sulphide, sodium sulphite, sodium sulphate, magnesium sulphate, and ferrous sulphide were selected for testing because they are salts of the more important minor elements necessary for plant growth and for comparison with the chlorine salts of the

¹ Manuscript received November 24, 1939.

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same metals (to be discussed in the next paper), and also because it has been shown that sulphur and chlorine in some chemicals can exert important influences on the sheath and on the exsheathment of larvae when free of faeces (21, 22, 23). For instance, some sulphides may alter the permeability of the sheath and might be of considerable importance in bringing the lethal chemicals into contact with the larvae. It has been shown that some sulphur compounds are more effective than analogous oxygen compounds against mosquito larvae, with the exception of diphenylene oxide (3). Ammonium sulphate (32), potassium sulphate (33), and nicotine sulphate (34) have been discussed in previous papers.

Copper sulphate has been tested relatively frequently against the free-living stages of bursate nematodes, especially in Germany. Some of the more recent papers include data comparable with those in this paper. In 1930 it was reported that a 1% solution rapidly made third-stage *Sclerostome* larvae motionless, and that even a 0.02% solution had this effect in a few hours; furthermore, on being removed to water they did not recover (29), a characteristic effect of copper sulphate, which had been previously noted (1). However, it was shown, about the same time, that against *Sclerostome* eggs and third-stage larvae in faeces, 1 and 2% solutions were not satisfactory (38). In 1934 it was reported that 1, 0.5, and 0.1% solutions were lethal to third-stage *Sclerostome* larvae in 15 to 30 hr., but that a 1% solution did not prevent the eggs maturing to the third stage, which a 2% solution did prevent. It was also reported that when a 0.1% solution was used on grass, about 50% of the larvae survived, but only 3 to 8% survived when a 1% solution was applied (12). It has since been stated that the addition of copper sulphate to kainit adds to the efficiency of the latter, and that under laboratory conditions a 4 : 1 kainit: copper sulphate mixture at the rate of about 300 lb. per acre killed all the eggs, but that out of doors the results were less promising (24).

Copper sulphate has also been tested against the eggs and larvae of *Stephanurus dentatus*. A 1 : 1,000 solution killed both free-feeding and infective larvae in 24 hr., and stronger solutions were more rapidly lethal. However, against infective larvae in mud, a 1 : 10 solution took 72 hr. to be effective when applied at the rate of 10 gal. per 100 sq. yd. (39), whereas a 1% solution at the rate of approximately 4 gal. per 100 sq. yd., although frequently applied, was not effective against either *Stephanurus* or *Oesophagostomum* larvae (40). A 12½% copper sulphate solution has been tested for 30 min. against *Anguillulina dipsaci* in sweet potatoes (20), and for two hours, as a 2, 1, and 0.5 solution against *Tylenchus* (= *Anguillulina*) *dipsaci*; in two hours the 2% solution was lethal, but not the weaker solutions (28). Under similar conditions, 2% solutions of ferrous sulphate, sodium sulphate, and sodium sulphite were not lethal (28). Copper sulphate and sodium sulphite were not lethal (28). Copper sulphate has also been reported as useless against *Heterodera schachtii* (4).

Copper sulphate applied to manure at the rate of one-quarter pound and one pound in one gallon (American) of water per cubic foot of manure killed about 60% of fly larvae; iron sulphate was not effective (6). Iron sulphate has been tested less frequently than copper sulphate against nematodes. As a 1% solution it did not prevent hookworm eggs from hatching, but killed the free-feeding larvae (35). At the rate of 1680 lb. per acre, ferrous sulphate did not control the cysts of the *Heterodera schachtii* (10, 11), but in other small scale experiments ferrous sulphate did control potato sickness caused by *H. schachtii*, although less rapidly than did ferric chloride (18).

A few other sulphur salts, such as magnesium sulphate, have been tested against various pests. Magnesium sulphate was reported to kill wireworm (*Agriotes mancus*) larvae in soil (17), but not to kill fly larvae in manure (7). Sodium sulphide as a 0.6% solution was stated to be effective against *Tylenchus dipsaci* in two hours (27).

Sulphur has frequently been tested for the control of plant nematodes; the results, like those with so many other chemicals, are difficult to compare and are contradictory. It has been stated that 400 to 600 lb. of powdered sulphur per acre, prevented root-knot of sweet potatoes, and that even 200 lb. was beneficial (36); 250 to 500 lb. per acre has also been recommended against root-knot (42). Sulphur, at the rate of one pound per bushel of manure, was effective against fly larvae (5). Mixed with formaldehyde, it was reported as being more effective than lime against *Heterodera marioni* (43), but alone was useless against *H. marioni* (2), and against *H. schachtii* (19, 25), even with dressings of 500 to 1000 lb. per acre (37). In greenhouses, although applied at the rate of 2600 lb. per acre, sulphur gave no benefit against root-knot (26).

Carbon disulphide and potassium xanthogenate (which on decomposition releases carbon disulphide believed to be the cause of the latter's toxicity (30)) have also frequently been tested against plant nematodes. Again the reports show how varied the results have been. In pot experiments, carbon disulphide was stated to be of no value (4). At a rate of 220 gal. (American) per acre, carbon disulphide was also reported to be of no benefit in greenhouses (26), and at 30 gal. did not affect materially the degree of infection of potatoes with nematode disease (9). It was, however, stated to be superior to calcium cyanide, calcium cyanamide, and quicklime (8), but less effective than formaldehyde (43). Applied as an emulsion, containing 68% carbon disulphide, further diluted with 50 parts of water to one of emulsion, one gal. (American) per sq. ft. was reported to be effective against *H. marioni* (14). At the rate of four pounds per 100 cu. ft. of soil, when the temperature was 77° F., carbon disulphide was completely effective against *Rhabditis lambdiensis*, and two and a half to three pounds per cubic foot killed most of those mushroom parasites (16).

In pots, carbon disulphide also reduced the number of cysts on potatoes (25). At the rate of 750 lb. per acre it decreased nematodes by 48% (13), while 13.0, 20.0, and 30.0 cc. reduced *H. marioni* in 12 kg. of sandy soil to

6.4, 5.2, and 0.61% respectively (15). In practice, it was reported to give satisfactory results (42), especially against nematodes on trees and shrubs (41).

Potassium xanthogenate has been shown to be more effective against *H. radicicola* than against *H. schachtii*; larvae of the former are killed by 0.35 gm. per 300 cc. (=2750 lb. per acre foot) in light soil (30). The vapours of potassium xanthogenate were reported to penetrate soil and paralyse larvae in three days, and in small-scale experiments, at the rate of 140 lb. per acre, to have controlled potato sickness (18).

Methods

Full descriptions of the technique used to obtain the data on the lethal values of chemicals against the free-living stages of Sclerostomes have been given in earlier papers (31, 33). The results obtained with the chemicals discussed in this paper were all obtained by the same methods. Cultures of 40 gm. of *fresh* (less than four or five hours old) horse faeces, were treated with the chemical to be tested, and left to develop in a dark constant temperature room at $26^{\circ}\text{C.} \pm 3^{\circ}\text{C.}$ for several days or weeks; the larvae were then extracted in funnels, from which they were drawn on the tenth day. This technique probably gives a very exacting test of the chemical, since the conditions are very suitable for rapid development of the larvae. However, some larvae may escape contact poisons by crawling up the walls of the glass container, or gases by remaining in small lumps of faeces. When the larvae reach the infective stage and then die, it is possible to recover only those on the outside of the culture or on the walls of the container.

Exact data are not available, but the sediment visible in the tap water used during the extraction process suggested that the quantity of impurities increased during the latter part of this work. These impurities occasionally amounted to about 0.01 gm. per 500 cc., chiefly consisting of iron carbonate.

The 9-in. extraction funnels held about 1600 cc., and approximately the same quantity is poured off and replaced during the extraction process. However, it is probable that the concentration of impurities was insufficient to affect the larvae, either by making them exsheath or otherwise injuring them.

Potassium Xanthogenate

Results

Potassium xanthogenate was tested dry and in solution; the results obtained are illustrated in Fig. 1.

It was tested dry in quantities ranging from 0.25 to 20% of the faeces by weight. The addition of 0.2 to 0.5 gm. considerably reduced the number of larvae. However, from some of the cultures to which 1.0 gm. or more was added, a few larvae, many dead, were recovered; from one of the cultures treated with 0.75 gm., equivalent to 1.87%, a few larvae were obtained; the other two cultures were sterilized.

A 1 : 2 aqueous solution was applied in quantities of 0.25 to 15.0 cc.; the addition of 1.5 to 2.0 cc. reduced the number of larvae recovered to a

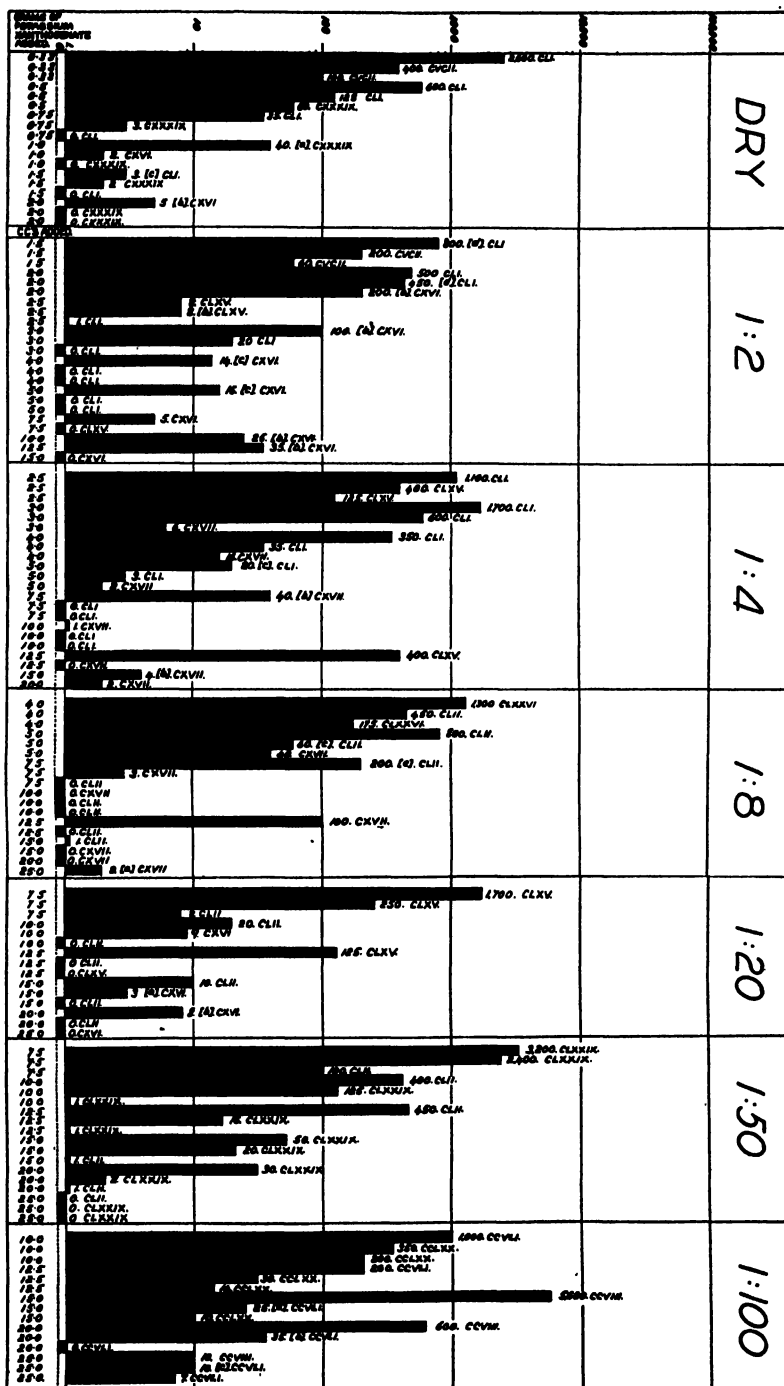


FIG. 1.

few hundred, and 2.5 cc. almost sterilized the cultures. However, from some of the cultures treated with 3.0 cc., and more, a few larvae, many of which were dead, were recovered. In 2.5 cc. of a 1 : 2 solution, there is almost 1.0 gm. of potassium xanthogenate, or 2.5% of the weight of the faeces.

The results obtained with a 1 : 4 solution, which was added in quantities of 0.5 to 20.0 cc., were also extremely irregular, but they suggest that 5.0 cc. will cause sterilization, although 400 larvae were recovered from one culture treated with 12.5 cc. In 5.0 cc. of solution of this strength there is slightly more than 1.0 gm., equivalent to 2.75% of the weight of faeces.

All the cultures receiving with 3.0 cc., or less, of a 1 : 8 solution yielded thousands of larvae. From two cultures treated with 5.0 cc. only 60 and 40 larvae were obtained; two of three cultures were sterilized by 7.5 cc. and all three cultures by 10.0 cc. One hundred larvae were recovered from one culture treated with 12.5 cc. In 10.0 cc. of a 1 : 8 solution there is also slightly more than 1.1 gm.

As a 1 : 20 solution, 2.0 cc. was the smallest quantity added. The results were again irregular; one culture was almost sterilized by 7.5 cc., 10.0 cc. was almost effective in three cultures, but 125 larvae were recovered from one receiving 12.5 cc. In 10.0 cc. of a 1 : 20 aqueous solution there is only slightly less than 0.5 gm., or 1.25% of the weight of the faeces in the cultures.

With one exception, all the cultures treated with not more than 7.5 cc. of a 1 : 50 aqueous solution contained thousands of active *Sclerostome* larvae. The addition of 10.0 cc., or more, considerably reduced the number of larvae in all cases. If the results, which were again irregular, are averaged, they suggest about 15.0 cc., containing 0.3 gm., equivalent to only 0.75%, as the approximate quantity necessary to sterilize the faeces.

A 1 : 100 aqueous solution was added in quantities ranging from 3.0 to 25.0 cc. All cultures receiving 5.0 cc., or less, yielded many thousand larvae. In two cultures, 7.5 cc. reduced the larvae to 700 and 550; in two, 10.0 cc., reduced them to 350 and 200. Those treated with 12.5 cc. yielded only 200, 30 and 14, but from one culture with 15.0 cc., 5600 larvae were isolated, and 600 from a culture treated with 20.0 cc. The other cultures receiving 15.0 and 20.0 cc. were practically sterilized, as were three to which had been added 25.0 cc., which contains about 0.25 gm., or 0.63% of the weight of the faeces.

Potassium xanthogenate was also tested as a 1 : 200, as a 1 : 300, and as a 1 : 400 aqueous solution; the results obtained are not illustrated. The 1 : 200 solution was added in quantities of 5.0 to 25.00 cc. All the cultures contained thousands of larvae with the exception of one treated with 20.0 cc. which contained 40, and those with 25.0 cc., which contained 500, 75, and 40 larvae.

One culture to which was added 25.0 cc. of a 1 : 300 solution contained only one larva, but the other cultures treated with a 1 : 300 solution and all treated with a 1 : 400 solution contained thousands.

These results indicate that potassium xanthogenate is most effective when applied as a weak solution; this seems to suggest that it may act at least partially as a contact poison, or, if its lethal property is in the gas that it liberates, that the penetrating power of the gas is extremely low.

Carbon Disulphide

Carbon disulphide was tested in quantities ranging from 0.025 to 25.0 cc. Fig. 2 illustrates how irregular were the results obtained. It is improbable that this irregularity could be ascribed to loss of the chemical from the containers as a gas, especially in those cultures that received the largest quantities as there was no odour of the gas in the constant temperature room. It can more probably be attributed to eggs or larvae or both in small lumps of faeces escaping its action.

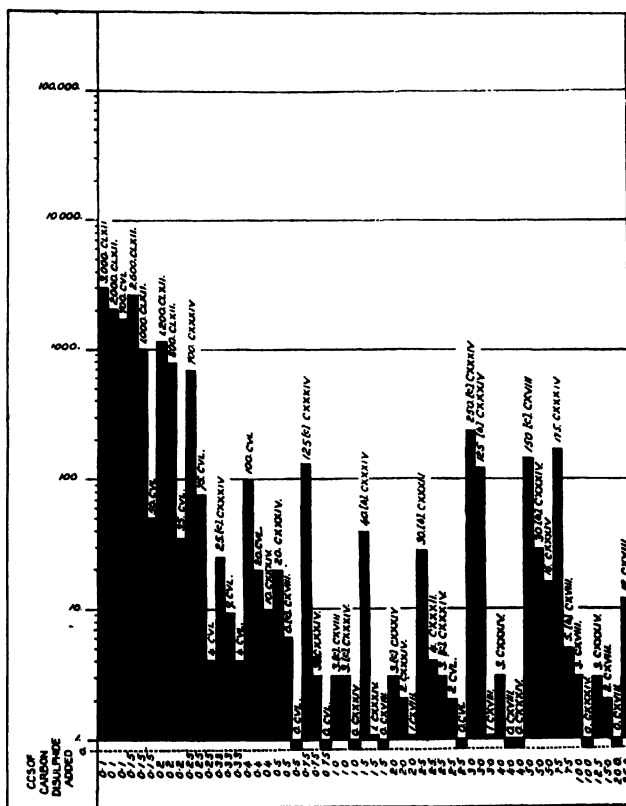


FIG. 2.

Even small quantities of carbon disulphide considerably reduced the number of larvae in most cases. The three cultures treated with 0.33 cc. were practically sterilized, as were a few with lesser amounts, but from two of the cultures receiving 3.0 cc., 250 and 175 larvae were obtained, including some dead, and from cultures treated with 5.0 and 7.5 cc., 150 and 175 larvae

were obtained. These results suggest that approximately 1.0 cc. will cause sterilization under conditions comparable with the technique used. If carbon disulphide gas is the lethal form against *Sclerostome* larvae, it is probable that, in containers filled by faeces, and if the gas would penetrate the faeces satisfactorily, it would be relatively more effective. However, the irregular results both with potassium xanthogenate and with carbon disulphide suggest that the characteristic of penetrating faeces, which would be so essential in practice, is lacking. The addition of 1.0 cc. of carbon disulphide is equivalent to adding approximately 3.1% of the chemical, by weight.

Zinc Sulphate

The results obtained with zinc sulphate are illustrated in Fig. 3.

This chemical, like other sulphur or chlorine salts of copper, iron, zinc, etc., frequently causes the death of the larvae after they have reached the third stage, although added to the faeces before eggs could have made much development. Whether these larvae, even soon after reaching the third stage, would have been capable of infecting a host is not known.

Zinc sulphate was mixed dry in cultures in quantities of 0.25 to 8.0 gm. When 0.5 gm., or less, was added, numerous larvae, although fewer than in the controls, reached the third stage and continued to live. When 0.75 to 1.5 gm. was mixed in the culture, many larvae reached the third stage, but a considerable proportion died comparatively rapidly. In one out of three cultures treated with 2.0 gm., numerous larvae reached the third stage but died subsequently; similar, but less marked, results were obtained when 3.0, 4.0, and 5.0 gm. were added. An average of these results suggests that about 2.5 gm., equivalent to 6.25%, is the amount of dry zinc sulphate necessary to cause sterilization.

Applied as a 1 : 2 aqueous solution 0.5 cc., or less, caused a comparatively slight reduction in the number of larvae. When from 0.75 to 4.0 cc. was added, numerous larvae were still isolated, but in most cultures many of these rapidly died. The addition of 5.0 cc. limited the number of larvae and also impaired their vitality. From one of three cultures treated with 7.5 cc. many larvae were isolated, but most of these soon died. The addition of 10.0 cc., or more, was effective in causing sterilization. In 7.5 cc. there is approximately 3.0 gm. of this chemical or 7.5% of the weight of the treated cultures.

When 5.0 cc., or less, of a 1 : 4 solution was added, numerous larvae were recovered from all the cultures; when 2.5 cc., or less, was added, the vitality of the larvae did not appear to be affected, but the addition of 3.0 cc., or more, had a marked effect on their viability. One hundred or more larvae were recovered from one of three cultures treated with 7.5, 15.0, and 20.0 cc., but many were dead. In 10.0 cc. of this solution there is about 2.25 gm. of zinc sulphate, or approximately 5.6% by weight.

Less than 5.0 cc. of a 1 : 8 solution did not have much effect on the vitality of the numerous larvae recovered; the addition of 7.5 cc., and more, reduced

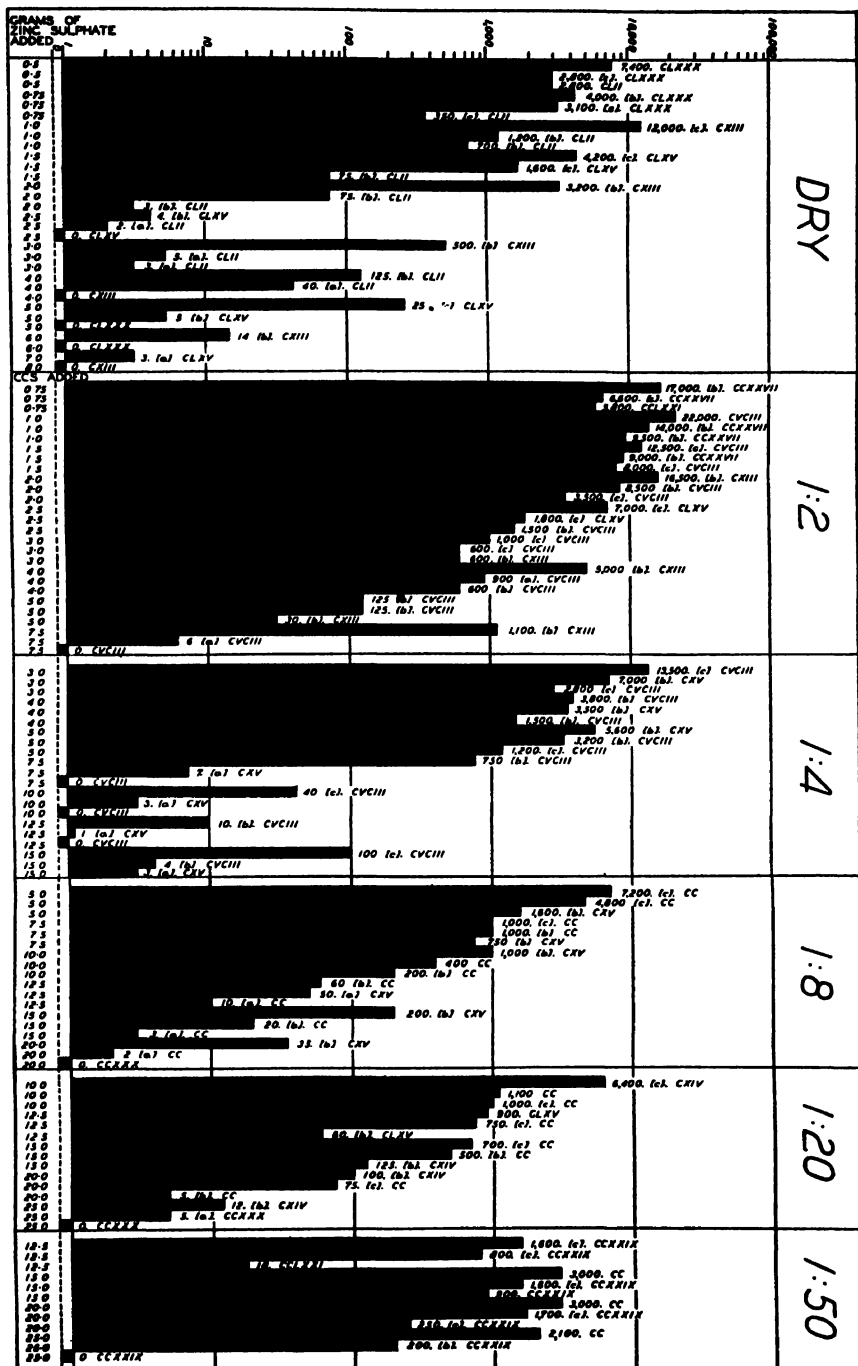


FIG. 3.

their numbers and viability. An average of the results obtained suggests that about 15.0 cc. of a 1 : 8 solution is necessary to cause sterilization. In this quantity there is about 1.8 gm., or 4.5% by weight.

As a 1 : 20 aqueous solution, 10.0 cc. was the minimum quantity that had a consistently marked effect on the numbers and vitality of larvae. The addition of 12.5, 15.0, and 20.0 cc. progressively reduced the numbers, but 25.0 cc., containing slightly less than 1.25 gm., equivalent to 3.1% by weight, is probably necessary to cause complete sterilization.

The results obtained with a 1 : 50 solution, which was tested in quantities ranging from 4.0 to 25.0 cc., were very irregular, both as to numbers and the viability of the larvae. One culture was completely, and another almost, sterilized by 25.0 cc., but from the third, 2100 active larvae were isolated. Smaller quantities of this solution, even 10.0 cc. or less, reduced the numbers of the larvae.

The fact that zinc sulphate is less effective dry, or as a strong solution, seems to suggest that to act on the larvae it must be in very intimate contact with them. Since many survive for some days or weeks after reaching the third stage and then die, it may also be suggested that the poison can penetrate

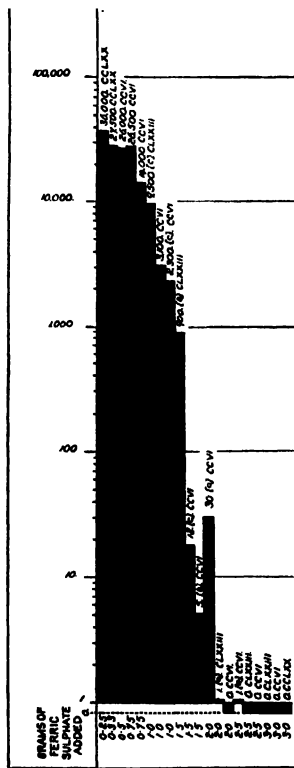


FIG. 4.

the sheath only slowly, and as third stage larvae do not feed in the free stage, it also suggests that it may not be necessary for the larvae to ingest the chemical to be killed by it.

Ferric Sulphate

Ferric sulphate was tested dry only; the results are illustrated in Fig. 4.

Many thousands of active larvae were recovered from the cultures receiving with 0.75 gm. or less. The addition of 1.0 gm. caused a slight reduction, whereas 1.5 gm. caused a considerable reduction in both numbers and viability of the larvae. When 2.0 gm., or 5%, was added no live larvae were recovered. One dead larva was recovered from a culture treated with 2.5 gm., but all the other cultures, treated with up to 8.0 gm., were free of larvae.

Ferrous Sulphate

Fig. 5 illustrates the results obtained with ferrous sulphate.

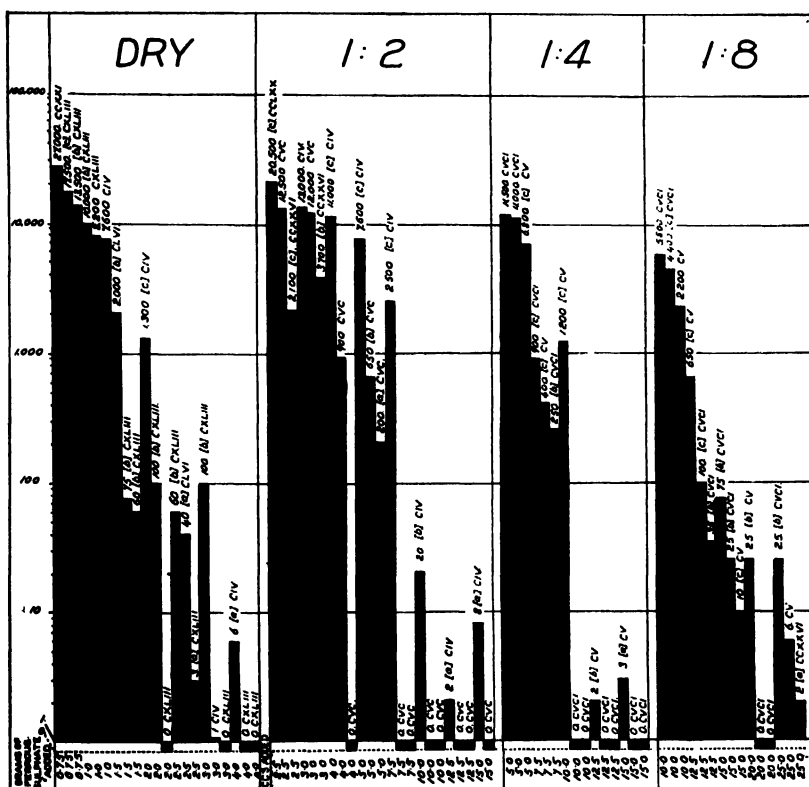


FIG. 5.

This chemical was applied dry in quantities ranging from 0.25 to 8.0 gm. Several thousand larvae were recovered from all cultures treated with 1.0 gm., or less, but some of the larvae obtained from one culture with 0.33 and with 1.0 gm., and from two with 0.75 gm., were dead. The addition of

larger quantities markedly affected the number and viability of larvae. Only from one of three cultures receiving 1.5 and 2.0 gm. were more than a thousand larvae obtained. All other cultures treated with 1.5 gm., and more, yielded only 100, or fewer, larvae—most, or all of which, were dead. An average of these results suggests that at most 3.0 gm., equivalent to 7.5%, is the quantity necessary to effect sterilization.

From one of three cultures treated with 7.5, 5.0, and 4.0 cc., and from all cultures with 3.0 cc., or less, of a 1 : 2 solution, thousands of larvae were isolated; however, some of those from some cultures receiving 2.5 cc., or more, were dead. All larvae from one culture treated with 5.0 cc., were dead, one with 4.0 cc. was free of larvae, as were two with 7.5 cc. The addition of 10.0 cc., and more, sterilized the cultures. This quantity of fluid contains approximately 4.0 gm., or 10% by weight, of the treated faeces.

A 1 : 4 solution was tested in quantities ranging from 2.0 to 25.0 cc. All cultures containing 5.0 cc., or less, yielded thousands of active larvae, with the exception of one treated with 5.0 cc., which yielded a few dead larvae. Three cultures treated with 7.5 cc. and one of the cultures with 10.0 cc. yielded many larvae, but some in each were dead. The others receiving 10.0 cc., or more, were sterilized. In 12.5 cc. of this solution there is almost 3.0 gm. of iron sulphate, or 7.5% by weight.

Applied as a 1 : 8 solution, thousands of larvae were recovered from all cultures to which 10.0 cc., or less, was added, and the vitality of most larvae seemed unaffected. When larger quantities were mixed in the faeces, both numbers and viability were markedly reduced; fewer than 100 larvae were recovered from one of the cultures treated with 12.5 cc. and from all receiving larger quantities. Two cultures were completely sterilized by 20.0 cc. and only a few larvae recovered from the third, as well as from the three treated with 25.0 cc.; most of these larvae were dead. In 25.0 cc. of this solution there is about 3.0 gm.

The results obtained by adding a 1 : 20 solution are not illustrated. It was tested in quantities of 5.0 to 25.0 cc. From all cultures, with the exception of one treated with 20.0 cc. and one with 25.0 cc., thousands of larvae were isolated; even from the two exceptions, 450 and 900 larvae were obtained. In the other cultures the vitality of only a few larvae appeared to have been lowered.

Cupric Sulphate

The results with cupric sulphate are illustrated in Fig. 6.

Very considerable differences exist in the quantities of this chemical necessary to free cultures of larvae and to cause many of the larvae to die after reaching the third stage. This makes an exact interpretation of the results more than usually difficult.

Dry cupric sulphate was added to cultures in quantities ranging from 0.25 to 10.0 gm. The vitality of the larvae in two of three cultures treated with 0.75 and with 1.0 gm., was affected, more especially with the larger quantity.

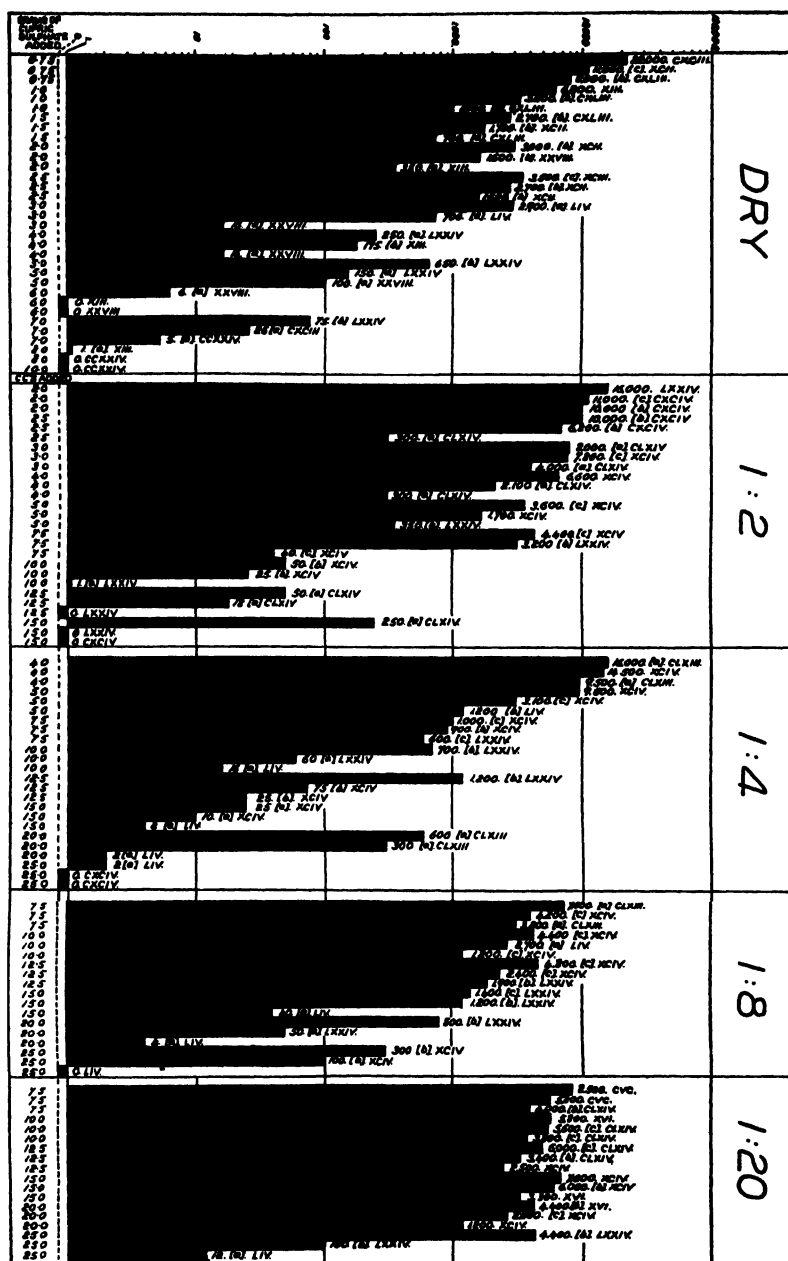


FIG. 6.

When 1.5 gm., or more, was added, some, or all, of the larvae from all the cultures were dead; yet some larvae were obtained from all cultures receiving 7.0 gm. or less, with the exception of two with 6.0 gm. All larvae were dead in one of three cultures treated with 1.0, with 1.5, and with 2.0 gm. and in two of three cultures with 4.0, with 5.0, and with 7.0 gm.; all the larvae

were dead, or the cultures free of larvae, when 3.0, 6.0, 8.0, or 10.0 gm. was added. On an average it is probable that about 4.0 gm., or 10% will kill the larvae, when added to fresh faeces.

A 1 : 2 aqueous solution was applied in quantities of 1.0 to 25.0 cc. A few of the numerous larvae recovered from one of the cultures treated with 1.5 cc. were dead. With the exception of one of three cultures receiving 2.0, 4.0, and 5.0 cc., all treated with 2.0 cc., and more, which yielded larvae, included dead larvae among them. In two of three cultures receiving 3.0 and 4.0 cc., the larvae were all dead; yet, in all cultures to which were added 5.0 and 7.5 cc., and in two of three with 10.0 cc., some were alive. No live larvae were found in any of the cultures treated with 12.5 cc. or more, whereas those treated with 20.0 and with 25.0 cc. were free of larvae. In 12.5 cc. of a 1 : 3 aqueous solution of cupric sulphate there is approximately 5.2 gm., or 13% of the weight of treated faeces.

When cupric sulphate was added as a 1 : 4 aqueous solution, 3.0 cc., or less, did not reduce the numbers or vitality of the larvae very considerably. From the cultures treated with 4.0 cc. many thousand larvae were recovered; however, those from two of the cultures were dead. The addition of 7.5 cc. considerably reduced both the number and viability of the larvae. The larvae from two of three cultures treated with 10.0 cc. were all dead, but a few were alive in all three receiving 12.5 cc. No larvae survived in any treated with 15.0 cc., or more; a few hundred dead larvae were recovered from two of three cultures treated with 20.0 cc. In 15.0 cc. of a 1 : 4 solution of copper sulphate there is slightly less than 3.5 gm., equivalent to approximately 8.7% by weight.

Added as a 1 : 8 solution, 4.0 cc., or less, did not affect the viability of the thousands of larvae recovered, and only a few were affected in two of the cultures treated with 5.0 cc. With a single exception over a thousand larvae were recovered from all cultures treated with 15.0 cc., or less; however, all the larvae were dead in one of three cultures receiving 10.0 and 15.0 cc., and in two of three with 7.5 and with 20.0 cc. In the others to which were added 7.5 cc., or more, there was a considerable loss of viability. An average of these results suggests that approximately 25.0 cc. of a 1 : 8 solution would be necessary to cause complete sterilization. In this quantity of solution there is 3.0 gm., equivalent to 7.5% of the weight of treated faeces.

When a 1 : 20 solution was applied to the faeces all cultures yielded thousands of larvae, with the exception of two of three cultures treated with 25.0 cc. Some of the larvae in about half of the cultures treated with 7.5 cc., or more, lacked viability. One of the cultures to which 25.0 cc. was added was sterilized; the larvae in one of the others were much reduced in numbers and viability, and many of the numerous larvae in the third also died. In 25.0 cc. of a 1 : 20 solution, there is slightly under 1.25 gm.

A 1 : 50 solution was also tested; thousands of larvae were recovered from all the cultures, but with the larger quantities a few of the larvae after reaching the third stage died. The results are not illustrated.

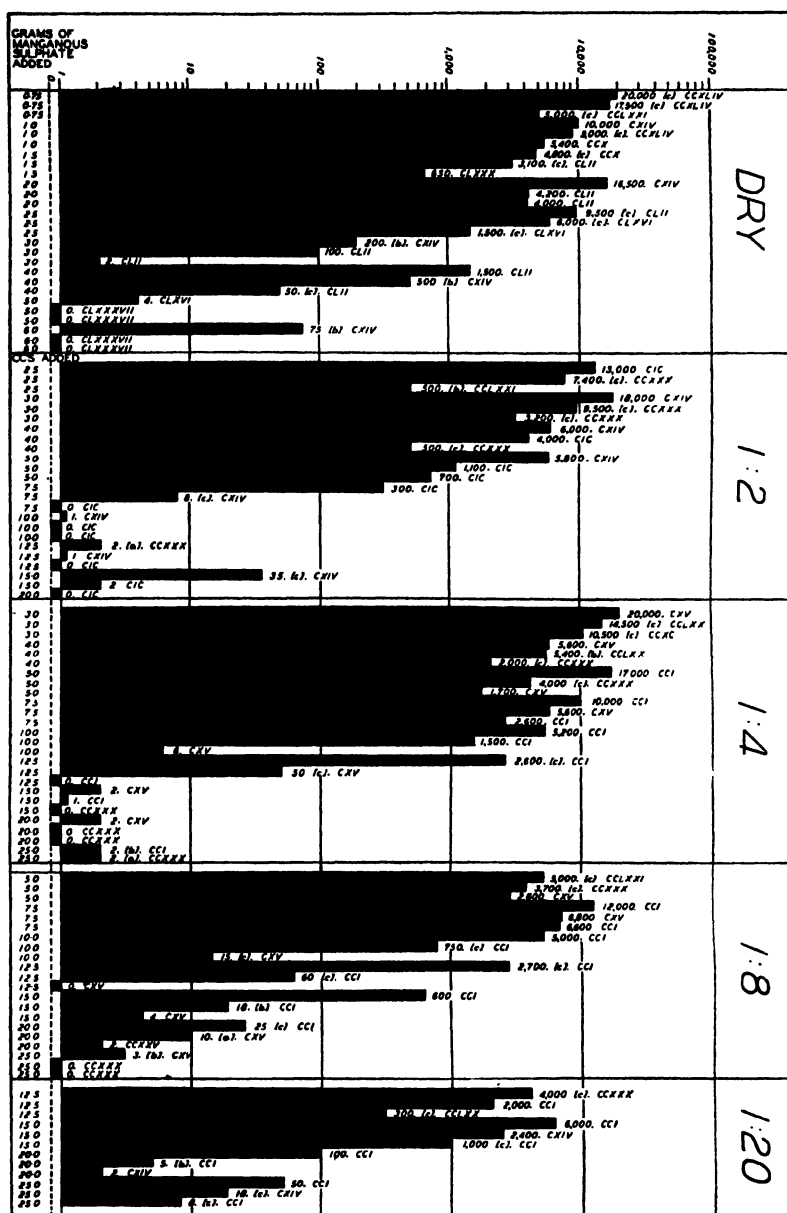


FIG. 7.

Manganous Sulphate

The effects of manganous sulphate are illustrated in Fig. 7.

Dry manganous sulphate was added to the cultures in quantities ranging from 0.25 to 10.0 gm. With the exception of one culture treated with 1.5 gm., which yielded 650 larvae, all those to which 2.5 gm. or less, was added yielded thousands of larvae; one culture to which 4.0 gm. was added also

yielded 1500 active larvae. A few of the larvae from all cultures treated with 0.75 and with 2.5 gm. were dead, as were a few from two of the cultures treated with 1.5 gm. and from one treated with 1.0 gm. The addition of 3.0 gm. markedly reduced the numbers and viability of the larvae, but the addition of 4.0 gm. was slightly less effective. Practically no larvae were recovered from the cultures to which 5.0 gm., equivalent to 12.5%, or more, was added, with the exception of one treated with 6.0 and one with 8.0 gm., from which 75 and 50 larvae were obtained, most of which were dead.

The minimum quantity of a 1 : 2 solution that was tested was 1.0 cc. Thousands of larvae were recovered from all the cultures treated with 5.0 cc., or less, with the exception of one with 2.5, 4.0, and 5.0 cc. However, some of the larvae in two cultures receiving 2.0 and 3.0 cc. and in one with 2.0 and 4.0 cc. were dead. One culture treated with 7.5 cc. yielded 300 active larvae, but the others with 7.5 cc., or more, were practically sterilized, with the exception of one treated with 15.0 cc. from which 35 larvae were obtained, of which a few were dead. These results suggest that, applied as a 1 : 2 solution, nearly 10.0 cc. containing over 4.0 gm., equivalent to about 10% is the necessary quantity to sterilize fresh faeces.

When added as a 1 : 4 solution, with the exception of one treated with 10.0 cc., which yielded only six larvae, all cultures treated with 10.0 cc., or less, and one treated with 12.5 cc., contained thousands of larvae; however, a few dead were included among those from one of the cultures treated with 2.5 and 5.0 cc., and from two treated with 3.0 and 4.0 cc. All cultures to which 15.0 cc., or more, and one to which 12.5 cc. was added, were practically sterilized. In 15.0 cc. of this solution there is nearly 3.5 gm. of manganous sulphate, or 8.75% of the weight of the treated faeces.

Applied as a 1 : 8 aqueous solution of manganous sulphate, 10.0 cc. was the minimum quantity that markedly reduced both the numbers and vitality of the larvae; in two of three cultures treated with this quantity some of the larvae were dead and their numbers were reduced to 750 and 15. The addition of 5.0 cc. also resulted in the death of some of the third stage larvae in two of the three cultures. One treated with 12.5 cc. was free of larvae, but, from the other two, 2700 and 60 larvae, including a few dead, were obtained. The addition of 15.0 cc. reduced the numbers in two of the cultures to 600 and 18, of which many were dead, and, in the third, to four. The cultures receiving 20.0 cc. were practically sterilized, and those with 25.0 cc. were completely sterilized; in the latter quantity there is about 3.0 gm., equivalent to 7.5%.

The addition of a 1 : 20 solution produced somewhat irregular results. From two of three cultures treated with 15.0 and with 12.5 cc. and from all treated with 10.0 cc., or less, thousands of larvae were obtained; however, from two treated with 12.5 cc. and from one treated with 10.0 and 15.0 cc., a few of the third stage larvae were dead. The addition of 20.0 cc. reduced the numbers to 100, 5 and 2; 25.0 cc. further lowered the numbers to 50, 18, and 8, including a few dead larvae.

Sodium Sulphide

Fig. 8 shows the results obtained with sodium sulphide. This chemical was tested dry in quantities ranging from 1.0 to 10.0 gm. The larvae in one of two cultures treated with 2.5 gm. were reduced in numbers and vitality, and 3.0 gm. had a more marked effect on the larvae of one of three cultures. The addition of 4.0 gm. reduced the larvae to 100, 50, and 14, and in the two last cultures, many were dead. When 5.0 gm., and more, was added, equivalent to 12.5% of the faeces by weight, the cultures were practically sterilized.

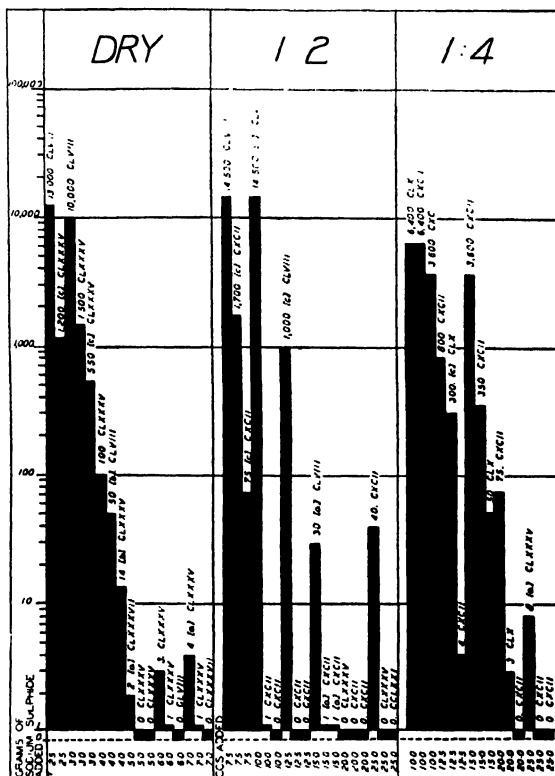


FIG 8.

The larvae in one culture treated with 5.0 cc. of a 1 : 2 aqueous solution were reduced to 1200. In two of three treated with 7.5 cc. they were reduced to 1700 and 75, a few being dead in both cultures. In one of three cultures treated with 10.0 cc. the larvae were reduced to one, and another culture was free of larvae. From the other cultures treated with 10.0 cc., or less, many thousands of larvae were recovered. One culture receiving 12.5 cc. yielded nearly a thousand larvae, of which a few were dead, one receiving 25.0 cc. yielded 40 active larvae, but the other cultures treated with these, and the intermediate quantities, were sterilized. An average of these results

suggests that about 12.5 cc., which is also equivalent to about 5.0 gm., or 12.5%, is the lethal proportion.

With the exception of one culture to which 3.0 cc. was added, all the cultures treated with 10.0 cc., or less, of a 1 : 4 solution yielded many thousands of larvae. The comparative lack of larvae in the exception, from which only just over 800 were isolated, can probably be ascribed to the presence of the parasitic fungus, *Harposporium anguillulae*, Lohde; in another culture, *Arthrobotrys obigospora*, Fres. was found, but it had not reduced the larvae to nearly the same extent, 15,000 being obtained.* The addition of 12.5 and 15.0 cc. of this solution considerably reduced the number of larvae. Two of three cultures treated with 20.0 cc. and all three treated with 25.0 cc. were sterilized. In the latter quantity there are approximately 5.5 gm., or 13.75% of the weight of faeces.

The results obtained with sodium sulphide as a 1 : 8 and as a 1 : 20 aqueous solution are not illustrated. With the exception of one culture treated with 20.0 cc. and of two with 25.0 cc. of the 1 : 8 solutions, and of one treated

*The writer is indebted to Dr. H. J. Brodie for the identification of these fungi.

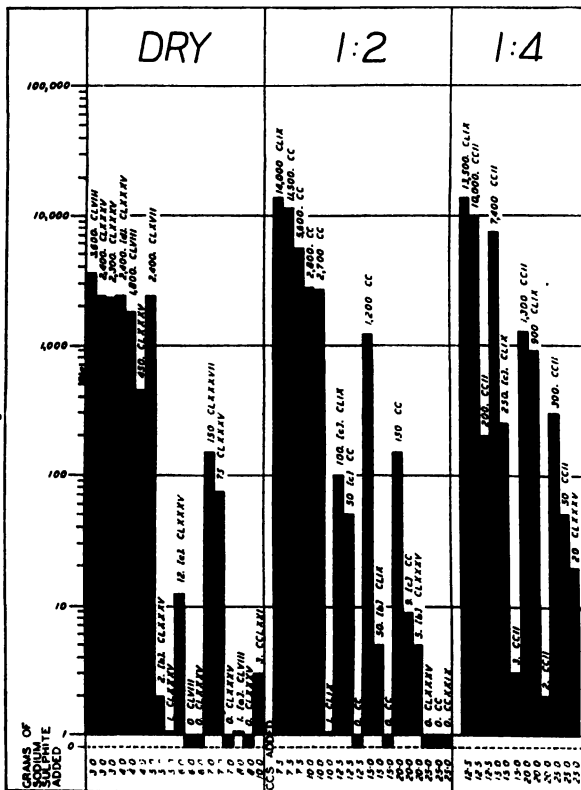


FIG. 9.

with 25.0 cc. of the 1 : 20 solution, all cultures yielded thousands of active larvae; from the exceptions, hundreds of active larvae were obtained.

Sodium Sulphite

The results obtained with sodium sulphite are illustrated in Fig. 9.

Thousands of active larvae were isolated from one of the three cultures treated with 5.0 gm., from two with 4.0 gm., and from all with 3.0 gm., or less. Two cultures receiving 7.0 gm. yielded 150 and 75 larvae, but the third culture was sterilized. Two treated with 5.0 gm., and all with 6.0, 8.0, and 10.0 gm., were practically sterilized. An average of these results suggests that 6.0 gm., or 15% is the lethal proportion.

One culture treated with 15.0 cc., two with 10.0 cc., and all with 7.5 cc., or less, of a 1 : 2 aqueous solution, yielded thousands of active larvae. One culture to which was added 20.0 cc. yielded 150 larvae; one receiving 12.5 cc. yielded 100 and another 50, which included dead larvae. The other cultures treated with these quantities were practically sterilized, one each by 10.0 and by 12.5 cc., two each by 15.0 and by 20.0 cc., and three by 25.0 cc. An average of the results indicated that almost 20.0 cc., containing 7.5 gm., equivalent to over 18%, is the approximate proportion to effect sterilization.

As a 1 : 4 solution, 12.5 cc. reduced the larvae in one culture to 200; 15.0 cc. reduced them in one culture to 250, including a few dead, and in another to three. The other cultures treated with these amounts, or with less, contained thousands of larvae. From the cultures treated with 20.0 cc., 1300, 900, and 2 larvae were obtained; 25.0 cc., which contains slightly under 6.0 gm. of sodium sulphite, reduced the larvae to 300, 50, and 20.

All cultures treated with a 1 : 8 and with a 1 : 20 solution, yielded many thousands of active larvae, with the exception of one of three cultures treated with 25.0 cc. of the 1 : 8 solution, which yielded only 20 larvae, and one of three treated with 25.0 cc. of the 1 : 20 solution, which yielded 1400 larvae, of which some were dead. The results with these solutions are not illustrated.

Sodium Sulphate

Fig. 10 illustrates the results obtained with sodium sulphate.

It was tested dry in quantities ranging from 1.0 to 14.0 gm. All cultures made with 4 gm., or less, contained thousands of active *Sclerostome* larvae. The addition of 6.0 and 7.0 gm. reduced the numbers of larvae and also the viability of a few of them. The numbers were further reduced in two cultures treated with 8.0 gm. and the third was free of larvae. One culture was completely and another almost sterilized by 10.0 gm., and most of the larvae obtained from the third were dead; this quantity is equivalent to 25% of the weight of faeces.

When a 1 : 2 solution was applied, one culture was sterilized by 15.0 cc. and all by 20.0 and by 25.0 cc.; those in which 15.0 cc. was used, and less, contained thousands of larvae. In 20.0 cc. of this solution of sodium sulphate, there is the equivalent of approximately 19% of the weight of faeces.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested; the results are not illustrated. The addition of 25.0 cc. of a 1 : 4 solution reduced one culture to 900 larvae, but all the other cultures made with this solution contained thousands of larvae.

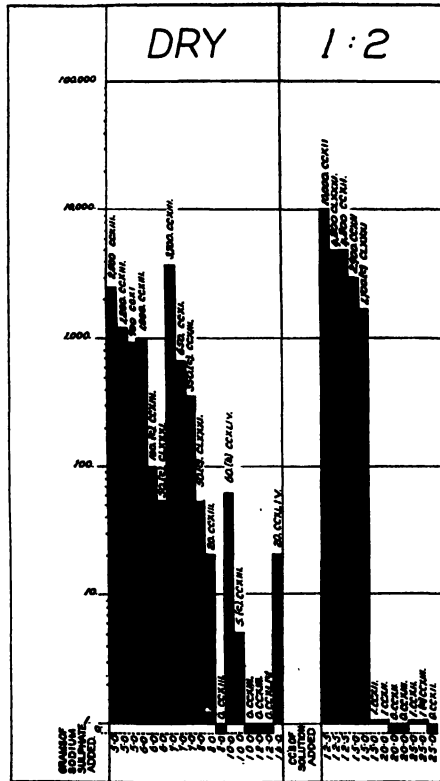


FIG. 10.

Magnesium Sulphate

The results obtained with magnesium sulphate are illustrated in Fig. 11.

Applied dry, all cultures treated with 6.0 gm. or less, contained thousands of active larvae; those treated with 7.0 and with 8.0 gm. also yielded many, although fewer active larvae. The addition of 10.0 and 12.0 gm. further reduced the numbers of larvae, while both quantities sterilized one culture. A few larvae were recovered from most of the cultures treated with 14.0 gm. (equivalent to 35% of the weight of the faeces), 16.0, and 20.0 gm., but all were dead, except those from one culture treated with 16.0 gm., which yielded 125 larvae.

All the cultures treated with 15.0 cc., or less, of a 1 : 2 solution yielded many thousands of larvae. Two cultures were sterilized by 20.0 cc. and two by 25.0 cc., but the third treated with 20.0 cc. yielded 350 larvae, some of

which were dead, and the third treated with 25.0 cc. (containing almost 10.0 gm. of magnesium sulphate, equivalent to 25%) yielded 2100 larvae.

A 1 : 4, 1 : 8, and 1 : 20 aqueous solution were also tested. Fewer larvae were found, of course, in the cultures treated with the largest quantities of fluid, but all the cultures contained thousands of active larvae. These results are not illustrated.

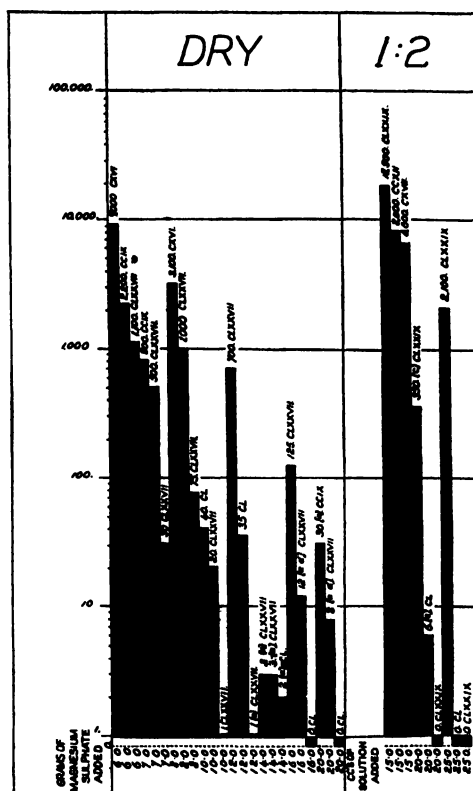


FIG. 11.

Zinc Sulphide

The results obtained with zinc sulphide which was tested dry only, are not illustrated.

All the cultures to which 10.0 gm. or less was added yielded many thousands of active larvae. The cultures treated with 12.0 and 14.0 gm. also yielded many thousands, most of them active, but in four of the five cultures, a few were dead. From one culture treated with 16.0 gm. only 100 larvae were obtained, many dead, but from the other two cultures treated with this amount, 41,000 and 2200 larvae were isolated, of which only a few were dead.

The addition of 20.0 gm. (50%) was more effective; one culture yielded only 30 dead larvae, another 200, of which a considerable proportion were dead, and the third 2200, including a few dead.

Ferrous Sulphide

Ferrous sulphide was tested dry only; it, like ferrous oxide, which was discussed in a previous paper (34), appears to have no lethal value. Ferrous sulphide was tested in quantities ranging from 1.0 to 20.0 gm. and compared with the controls there appeared to be no significant decrease or increase in the number of larvae isolated, or in their vitality.

Sulphur

Flowers of sulphur was tested in quantities ranging from 1.0 to 20.0 gm.; quantities of 10.0 gm., or less, were tested in duplicate, those over 10 gm., in triplicate.

TABLE I
CONTROLS FOR CULTURES TABULATED IN FIGS. 1-11

Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date culture made	Days kept in C.T. room	Average number of larvae isolated
	1935				1936— <i>Conc.</i>		
XIII	7 May	31	11,000	CLXXIII	26 November	60	37,000
XVI	22	22	25,000	CLXXVI	5 December	52	71,000
XXIII	9 July	16	93,000	CLXXVII	5	58	33,000
XXIV	9	18	48,000	CLXXIX	8	58	98,000
XXVI	9	18	70,500	CLXXX	9	57	69,000
XXVIII	11	19	2,700	CLXXXI	9	57	72,000
LIV	10 December	17	6,200	CLXXXV	23	54	16,000
	1936			CLXXXVII	30	48	11,000
LXXIV	24 January	24	11,500		1937		
XCII	11 March	22	26,000	CXCII	8 January	55	12,000
XCIII	19	14	27,000	CXCIII	11	45	35,000
XCIV	19	21	35,000	CXCIV	12	51	31,000
CIV	7 April	23	21,500	CVC	12	55	22,500
CV	7	24	19,500	CVCI	19	48	29,500
CXIII	1 May	22	70,000	CVCH	20	47	31,000
CXIV	1	22	33,000	CVCIII	22	52	37,000
CXV	1	37	13,000	CIC	26	48	50,000
CXVI	7	15	36,000	CC	27	50	26,500
CXVII	7	23	26,000	CCI	28	56	40,000
CXVIII	12	21	2,000	CCII	29	48	19,000
CXXXII	12 June	22	8,500	CCVI	12 February	52	36,000
CXXXIV	17	37	40,000	CCVIII	18	49	55,000
CXXXIX	9 July	18	43,000	CCIX	18	46	40,000
CXLIH	31	74	18,500	CCX	23	44	35,000
CXLIV	31	77	13,500	CCXI	24	50	32,000
CVL	3 August	81	22,500	CCXII	1 March	38	51,000
CL	6	99	4,600	CCXIII	2	48	62,000
CLI	7	98	1,100	CCXXI	16	23	26,000
CLII	7	108	5,000	CCXXIV	17	65	41,000
CLVI	26 October	39	14,500	CCXXVI	30	48	34,000
CLVIII	29	43	39,000	CCXXVII	1 April	51	42,000
CLIX	30	44	71,000	CCXXIX	5	56	33,000
CLX	2 November	42	15,500	CCXXX	6	55	22,500
CLXI	5	49	39,000	CCXLIV	20 May	25	49,000
CLXIII	9	45	55,000	CCVLI	21	34	29,000
CLXIV	11	43	24,500	CCLXX	22 July	74	34,000
CLXV	12	53	43,000	CCLXXI	23	83	16,000
CLXVI	16	45	23,500	CCXC	28 October	28	46,000

Sulphur appears to have no value in reducing the numbers of larvae. In a few cultures made with various quantities, a few of the recovered larvae were dead, but there appeared to be no correlation between the amount applied and the subsequent death of the larvae. In fact, the results suggest that by controlling parasitic fungi or other harmful factors, the number of larvae recovered may be increased. In 20 of the 30 cultures more larvae were recovered from the treated than from the control cultures. The controls were those for series XIII, XXIII, XXVI, CXLIV, CLXV (see Table I). Five cultures contained more than three times as many larvae in the treated as in the control cultures, and ten, more than twice as many; naturally, the largest increases tended to be in the series with the fewest larvae in the controls—13,500 and 11,000. The average number of larvae in the treated cultures was 42,400, an increase of just over 25% of the number that the controls show, would have been in 30 untreated cultures.

Conclusions

Potassium xanthogenate, unless its cost were too high, might be used under some conditions as a lethal agent against the free-living stages of *Sclerostomes*, since it is fairly effective as a weak solution. Carbon disulphide, unless it proves more effective as an emulsion, does not appear to have sufficient penetrating power. The other salts discussed in this paper all seem too weak to be of value alone. The "delayed action" of some, however, suggests that, if they were brought into intimate contact with the larvae through the action of another chemical, they might have some value. Unfortunately, much more work must be done before any constructive proposals can be made on this basis.

Acknowledgments

It is a pleasure to be able to add the names of Messrs. Rohm and Hass Co. Inc., of Philadelphia, and of Messrs. The Dow Chemical Co., of Midland, Michigan, to the list of firms who have given chemicals for this work, and to be able to record the writer's gratitude to them.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 18, SEC. D.

DECEMBER, 1940

NUMBER 12

PARASITES OF SOME CANADIAN SEA MAMMALS¹

By L. L. Lyster²

Abstract

Four nematodes, two acanthocephalids and specimens of the cestode genus *Diphyllobothrium* are reported from seals and white whales in Arctic and temperate coastal waters of Canada. *Phocascaris nelsiki* sp. nov. is described from *Phoca hispida* and observations on flagella in *Anisakis simplex* are recorded.

To the greater number of Canada's Arctic and sub-arctic inhabitants the sea-mammals supply staples both of food and clothing. In the course of our survey of parasites a considerable amount of material has been collected post-mortem, from these animals. Seals are represented by collections from several of the eastern Arctic posts. This material (as was the single parasite-free walrus examined) was secured chiefly with the co-operation of the Royal Canadian Mounted Police and the Hudson's Bay Company who sent formalized viscera to the Institute. In addition, collections were made by Dr. Parnell in 1934 (10) and by the author in 1939. White whale parasites, collected in the Gulf of St. Lawrence, were supplied to us by Dr. V. D. Vladymov and viscera of whales have also been received from the eastern Arctic posts.

Formalization of viscera leaves much to be desired from the standpoint of the condition of the material collected, but it has made available resources and collecting points that would not otherwise have been reached. The sources of the present material, as listed with the hosts, have been mapped in an earlier paper (4).

Parasites recovered during the survey included nematodes, cestodes and acanthocephalids. Trematodes are conspicuously absent; our correspondence files include reference to "liver flukes" of seals at Cape Smith, but our post-mortem studies on livers from this area, though showing fatty degeneration and necrosis, have produced no trematodes. Personal notes also include a report by a Cape Smith native who described white rough livers in local seals, a condition which, according to the Esquimaux, renders the entire carcass unfit for food.

In fish-eating seals and white whales, nematodes invariably occurred in the stomach in close association with the partially disintegrated portions of fish

¹ Manuscript received June 8, 1940.

Contribution from the Institute of Parasitology, Macdonald College, Que., with financial assistance from the National Research Council.

² Graduate assistant. (Now deceased.)

found there. In many cases these lodged so firmly in the fish muscles that it was necessary to dissect them out. These nematodes are adult forms and therefore not introduced with the food. Whether they feed on the fish ingested by their host, act as symbionts serving to break up the bolus, or whether there is some more obscure relationship, remain interesting speculations. Extracorporal digestion among ascarids has been noted by Hoeppli (7) among *Contracaecum* sp. in seal stomachs. The nematodes he studied were actively parasitic on the host, but the fact is established that they are capable of producing histolytic changes in an external medium. The nematodes concerned must be highly anti-proteolytic; apparently healthy individuals are to be found in the oesophagus, stomach, and small intestine of the more heavily infected animals.

Nematodes

Three species of different genera are included in our seal collections and one species is reported from white whales.

Porrocaecum Railliet & Henry, 1912.

The most common parasite of the bearded seal belongs to the genus *Porrocaecum* on the criteria of the presence of a single intestinal caecum and dentigerate lips. The single species of the genus reported from Arctic seals has been discussed by Baylis (1) who reviewed the earlier studies and material and differentiated the species largely on the structure of the lips and alimentary tract. Since establishment of the genus, many major points of species differentiation have become generic and it is difficult to refer the present material to the species *P. decipiens* described by Baylis; a detailed description is accordingly given.

The male is 9 to 11 mm. long by 0.5 to 0.6 mm. wide in greatest measurement. The female is about 15 mm. long by 1 mm. wide, with a maximum specimen of 37 to 1.25 mm.

The two subventral lips are triangular in shape, not unlike a conventional heart, apex toward the base, anterior margin cut medially to make two prominent lobes. The pulp of the lip follows the same outline, leaving a semitransparent margin. A prominent single papilla is situated at the apex of the triangle. The anterior margin of the dorsal lip is similarly cleft, but the base of the lip is wider than the free portion. About midway the length of the dorsal lip there is an external thickening behind which two papillae are situated. No interlabia are present. Dentigerous ridges vary in arrangement and extent. In some specimens they are to be seen only at the contact of the anterior lobes; in others they are continuous throughout the anterior margins while in one case they outline the lateral margins of each lip. In some specimens it was impossible to find any evidence of dentigerous ridges, though other features were constant. These structures were never very conspicuous.

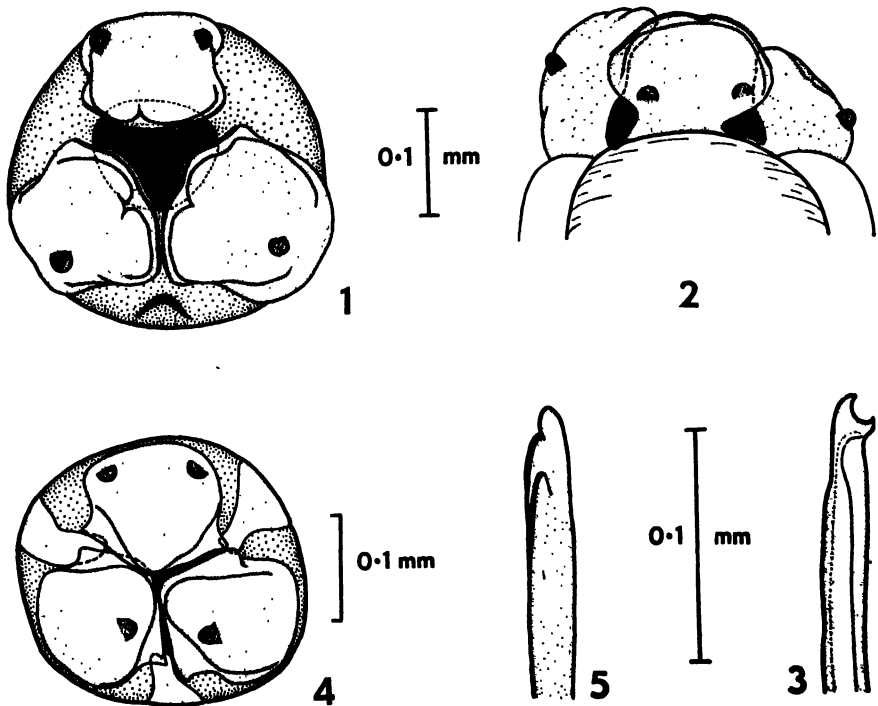
The oesophagus is divided into the usual two structurally different parts, continuous with each other, and opening without change of direction into the

intestine. The anterior portion is 1.7 mm., the posterior 0.15 mm., in length. At the point of origin of the intestine rises a diverticulum which runs anteriorly to a point approximately in line with the limit of the glandular oesophagus, a distance equal to about one-fortieth the body length.

The female tail is bluntly rounded with a small terminal knob. The vulva is one-third, or somewhat less, the body length from the anterior end. The genital structures are as figured by Baylis.

The male body terminates in a sharp point but is given a blunt appearance by a cuticular wing, 0.03 mm. at its widest point, which is continuous around the end of the body, from a point roughly in line with the cloacal opening. The tail is 2.5 mm. long carrying seven papillae; three are lateral and paired, one is medial and almost terminal. More or less parallel with the spicules four pairs of papillae are situated on either side of the cloaca and a single large one is central and just anterior to this opening. The greatest number of pre-anal papillae found in any of our specimens was 52 pairs. For some distance anterior to the cloaca they occur in a regular line parallel to the sides of the body, but about the thirtieth they tend to become scattered into a general plan of two rows in which the papillae alternate in pairs.

The spicules are equal, 1.5 mm. long. They are straight to the tip, with lateral alae which curve ventrally, meeting just before the end. Terminally



FIGS. 1-3. *Porrocaecum decipiens* ex *E. barbatus*. 1. En face view. 2. Head, dorsal view. 3. Spicule. FIGS. 4-5. *Contracaecum osculatatum* ex *P. vitulina*. 4. Head, en face view. 5. Left spicule.

the spicule broadens and a semilunar indentation gives it a clawed appearance.

These specimens are notably smaller than those discussed by Baylis. This is a fact for which it is difficult to find an explanation; even although no well-developed eggs were noted, it seems unlikely that the increase in size to complete maturity, would be as much as two to three times. Relative measurements, a more satisfactory criterion, correspond with those given by him.

The main lips are not identical with those discussed by Baylis and there is variation in the numbers of pre-anal papillae; these two factors are considerably influenced by fixation within the limits of the present differences.

Because of general head structure, papillary arrangement and, notably, on biological grounds, the present material has been considered to be the species *P. decipiens*. The variations noted are not of sufficient significance to allow specific differentiation and Arctic seals are considered as carrying the single species.

Less mature specimens of the same species were recovered from the stomach of most of the individual animals studied. These were usually attached to the mucosa in bundles of about 20 worms. The largest specimen occurring in this site measured 1.8 by 0.08 mm.; the oesophagus was 0.28 mm. and the caecum 0.04 mm. in length. The larger number of these forms was about half this size. Specimens found unattached in the stomach included many enclosed in their larval sheath, and adults were also recovered here. A single specimen was taken from the oesophagus of a Jar Seal (*Phoca vitulina*).

Most specimens of *Erignathus barbatus* that we have examined are parasitized by this nematode; these have come from ports on either side of Hudson Strait. In addition, we have records of it from *Phoca groenlandica*, Stupart Bay, from "Seals", Havre St. Pierre, Que., and Craig Harbour, and from a "Jar Seal" taken at Wolstenholme. Because of feeding habits, etc. of *P. vitulina* the "Jar Seal" would seem to be a mistaken identification of the host.

Contracecum Railliet & Henry, 1912.

This genus has been fully discussed by Baylis (3), who lists three species from pinniped hosts. The present material corresponds most closely to his description of the northern species *C. osculatum*. The dorsal lip is of relatively uniform width with sharp cuticular lobes, the oesophagus is cylindrical, the spicules over nine mm. long; these are features on which he bases the identification. In over-all measurements and in most details of structure the present specimens correspond exactly with that species. Some differences in detail, however, must be noted.

In the male specimens, averaging 40 mm. long by 1.8 mm. wide, in no instance were there as many as 50 pre-anal papillae; they numbered about 40. Wrinkling and shrinking of the cuticle obscures these structures and will account for at least some of the difference. The spicules were slightly longer than Baylis records, ranging up to 10.3 mm. The spicular alae do not reach the end of the spicule and that on the right does not extend so far as that on the left.

In the female, up to 60 mm. long by 2.5 mm. wide, the vulva is situated at about the limit of the anterior sixth of the body. The oviduct divides, each portion entering a branch of the uterus, at about one-third the body length. Near this point the ovaries rise and proceed backwards to within 9 mm. of the posterior end (in a 60 mm. specimen), then run forward about 5 mm. to meet the uterine tubes.

In spite of the variations (which are readily attributable to different methods of fixation) since over-all characteristics and particularly the structure of the lips, are so closely identifiable with Baylis's detail for *C. osculatum*, it is to this species that the present material has been assigned.

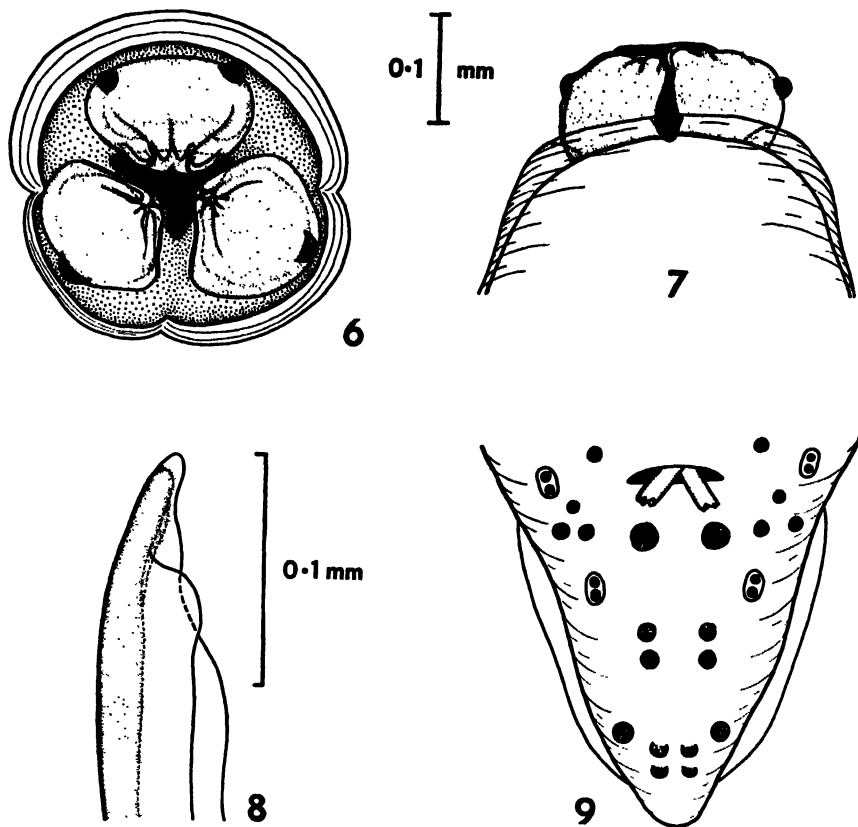
The species has been recorded in the survey from *P. hispida* and *P. vitulina*. It is found from Dundas Harbour, Devon Island, and Craig Harbour in the north to Havre St. Pierre, Que., in the south. Intermediate points of collection have been Lake Harbour, Clyde River in Baffin Island, and Stupart Bay and Wolstenholme on Hudson Strait.

Phocascaris Höst, 1932

This genus has hitherto included the single species *P. phocae* reported by Höst from *P. groenlandica* (8). It is very like *Contracaecum* in conformation of alimentary tract but because of modified and dentigerate lips it cannot be placed in that genus. *Phocascaris*, accordingly occupies a valid place among the Anasikinae.

Several specimens collected from seals in the Canadian north show the features that characterize this genus. None of them, however, conform exactly to Höst's description of the species (8). In *P. phocae* the anterior margins of the lips are formed by four labial folds decorated with dentigerous ridges and each subventral lip carries a large and a small papilla. In the present material the anterior margins of the lips are entire with an occasional exception in the case of the dorsal lip when a medial indentation may occur. Dentigerous ridges are continuous from about the midlateral areas of each lip. Somewhat removed from the margin, six labial folds are conspicuous, the outermost pair being closely applied and outlined by dentigerous structures giving the effect of four submarginal scallopings. A single large papilla is to be seen on each subventral lip and a pair of double papilla lateral to the male cloaca are further features that distinguish this material from *P. phocae*, in which no comparable structures were seen. These differences and the size are sufficient to make it impossible to identify these specimens as belonging to Höst's species, and it is accordingly necessary to create a new species to designate our collection. For this the author proposes the name *P. nelsiki* sp. nov.

The female is 30–76 mm. long with a maximum width of 0.6 to 1.2 mm.; male specimens measure 20 to 51 mm. long. The oesophagus, about one-tenth to one-fifteenth the body length, often coils upon itself midway in its course. The proventriculus about one-eighth the length of the oesophagus proper, opens without change of direction into the intestine. An intestinal



FIGS. 6-9. *Phocascaris netsiki* ex *P. hispida*. 6. En face view. 7. Head, ventral view. 8. Spicule. 9. End of male, showing peri-cloacal and terminal papillae.

caecum is equal to or only slightly greater than the proventriculus. A wide oesophageal diverticulum runs posteriorly a distance equal to half the length of the oesophagus proper, terminating in a sharply recurved distal point. Inconspicuous lateral cephalic alae occur.

The female genital complex varies in organization from that described for *P. phocae*. The oviduct opens by a narrow constriction into a Y-shaped uterus. The two posterior branches of the uterus are joined by the much smaller ovaries which enter dorsally and just short of the ends of the branches. The ovarian tubes follow a convoluted course posteriorly, almost to the anus, then run directly forward to the level of the oviduct. The vulva is situated about one-fifth the body length from the anterior end. The eggs are spherical or slightly ovoid, thin-shelled, containing a segmented ovum; they are about 0.08 mm. in diameter.

The male spicules are long, sub-equal and simple. They are alike in structure. A strongly chitinized dorsal rod is continuous ventrally with a semitransparent hollow tube. This latter divides about 1 mm. from the end to form two lateral alae. One of these is extended around the end, the

other is discontinued just anterior to the end of the spicule. In a specimen 51 mm. long the left member was 5.0 mm. and the right 5.5 mm. long. They average about one-tenth the body length. Five pairs of single and one pair of double papillae decorate the tail and a similar number are peri-cloacal. of the last a pair of double papillae are laterally in line with the cloaca. Fifty pairs of single pre-cloacal papillae follow a regular course parallel to the sides of the body. Narrow lateral caudal alae are present.

In both sexes the cuticle is finely striated throughout; these striae coupled with close cuticular folds, form a distinct collar around the base of the lips.

Infections with *P. netsiki* were never heavier than five to ten worms in a host. They were found in *Phoca hispida* from Craig Harbour, Clyde River, and Lake Harbour, N.W.T., and in a "seal" from Cape Smith, N.W.T.

Anisakis Dujardin, 1845

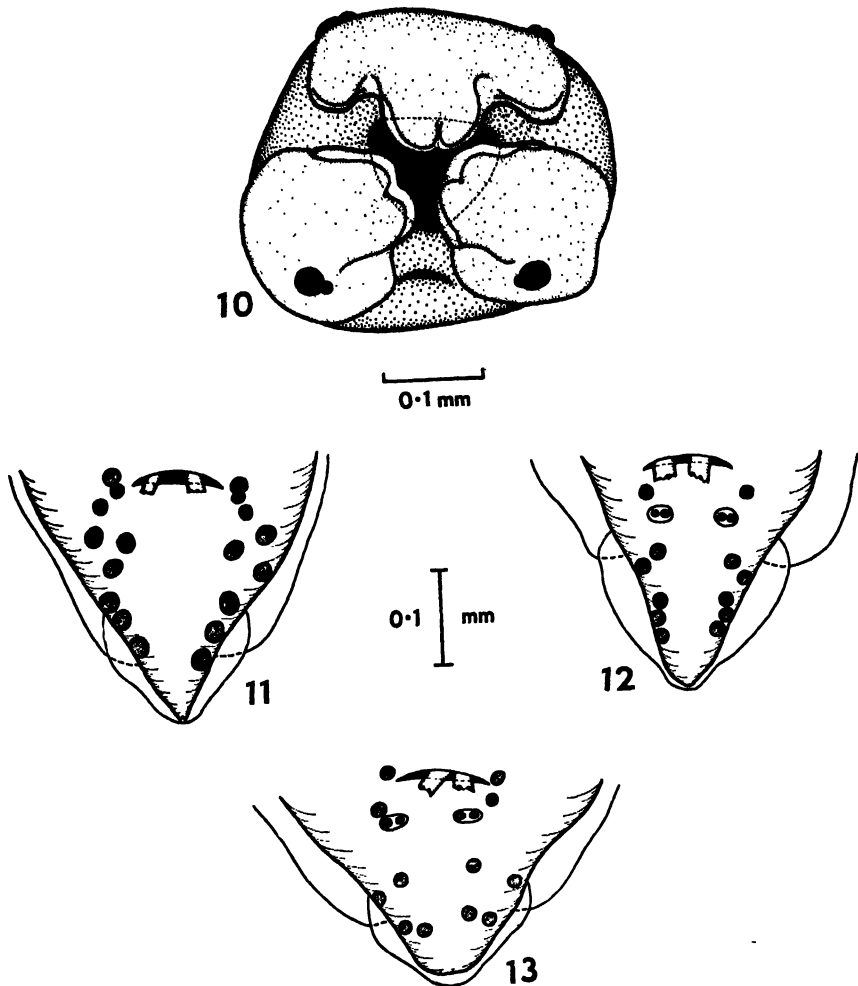
Several thousand ascarids collected from the alimentary tract of *Delphinapterus leucas* are identifiable as belonging to the genus *Anisakis*, on a basis of absence of alimentary diverticula and interlabia and presence of dentigerous ridges and a two-part oesophagus.

From marine mammals eight species of this genus have been described, but marked confusion of early descriptions and later synonymities make specific determination almost impossible. Three species, *A. similis*, *A. rosmari* and *A. physeteris* have been reported from carnivorous mammals, and one, *A. insignis*, from a freshwater dolphin. The remaining four species have been described from various porpoises and small whales, and it is among these that most confusion has arisen. *A. dussumierii* Van Beneden, 1870, was first described as *A. simplex* by Dujardin; *A. typica* Diesing, 1860, has been listed as a synonym of *A. dussumierii* by Stiles and Hassall (11) and *A. kukenthalii* Cobb, 1888, has been proposed as a synonym of *A. simplex* Rudolphi, 1809, by the same authors.

These four species are not only poorly differentiated, but they have a great deal in common. The problem of assigning the present material to a place in the genus is one of selecting constantly differential characteristics and interpreting them to apply to our specimens. Table I, correlating the measure-

TABLE I
COMPARATIVE MEASUREMENTS OF *Anisakis* FROM MARINE CETACEA (MM.)

Male	Female	Oesophagus I	Oesophagus II	Spicules	No. post. papillae	No. prepapillae	Width striae	Species
31-70	37-90	4	1.25	3.9 and 0.96	6-10	75 plus	32 μ	<i>typica</i>
79	70-100	5.5	1.5	2.7 and 1.5	8-10	?	30 μ	<i>dussumierii</i>
37-130	79-200			1.68	6-8	50 plus	23 μ	<i>simplex</i>
70-90	80-100	5.1	2.5	2.3 and 1.7	7-8	90	30 μ	<i>kukenthalii</i>
60-90	63-110	5.4	1.6	2.0 and 1.4	7-9	56-90	23 μ to 38 μ	Present form



FIGS. 10 - 13. *Anisakis simplex* ex *D. leucas*. 10. En face view. 11 - 13. End of male showing progressive modifications in papillary arrangement.

ments given for cetacean-parasitizing *Anisakis* in the literature (notably, in Stiles and Hassall (11)), illustrates the inadequacy of measurement in specific diagnoses. (A similar table is used by Baylis (2) in establishing his species *A. physeteris*). The minimal measurements of 31-79 mm. in the male and 37-80 mm. in the female and the maximal measurements of 70 to 130 mm. and 90 to 200 mm. in the female will not exclude a single species. Similarly measurements of structures and papillae counts are non-differential. If any species is in any way distinct it is *A. typica*, on a basis of insignificantly smaller size, and its separate identity from *A. dussumierii* has already been questioned. In the same way, arrangements of structures do not offer a means of separation. No detail of labial structure has been held significant except the tendency for greater distinction between the basal and lobate portions of the dorsal lip illustrated by Krabbe (9) and by Stiles and Hassall working with "*A. typica*".

The female worms of the group are undifferentiable from descriptions. The variations in spicule length and papillae arrangement in the males are limited to differences within definable limits into which all species will fall.

Because they are found in a closely related group of circumpolar animals of similar habit, and because there seem to be no satisfactory morphological criteria for recognizing distinct species, it seems unlikely that these four members of the genus are dissimilar beyond the insignificant variations that are shown within most nematode species. This view is further borne out by the considerable differences that have been noted between these four members of the genus on the one hand and *A. similis*, *A. physeteris*, and *A. rosmari* on the other, a differentiation that is to be anticipated on biological and ecological, quite apart from morphological grounds.

For these reasons the present material has been assigned to the species *A. simplex* which is here considered to have as synonyms *A. kukenthalli*, *A. typica*, and *A. dussumierii*.

Males in our collection measure 60 to 90 mm. by 1.7 to 2.4 mm., females 63 to 100 mm. by 2.0 to 2.6 mm. The oesophagus has an anterior muscular portion averaging about 5.5 mm. long, and a posterior, usually laterally sigmoid, proventricular portion 1.5 mm. long, or slightly greater. Cuticular striae 23 to 38 μ apart are continuous throughout the body length. The three lips follow the usual typical pattern; a crescentic dorsal lip 0.24 by 0.12 mm. bears a pair of anterior projections medially; two subventral lips are more nearly round in outline, with a pair of lobes reduced in extent but similar to those of the dorsal lip. Dentigerous ridges outline the opposed margins of these lips. The dorsal member carries a pair of papillae at the lateral corners and each subventral lip is decorated with a pair of ventral papillae. A pair of cervical papillae is about 1.0 to 1.5 mm. removed from the base of the lips.

The vulva of the female opens just in front of the middle of the body (45 mm. from anterior end in a specimen 105 mm. long). An oviduct about 12 mm. long loops longitudinally before entering the bicornate, posteriorly directed uterus about 33 mm. long. Each uterine branch is joined by a long, slender and highly convoluted ovary which runs backward for a short distance, then to the forepart of the body well anterior of the vulva. Intrauterine eggs are subspherical, 0.045 to 0.5 mm. in diameter.

The male spicules are slightly curved, slender, dissimilar; the left is 2.0 mm. or slightly more, the right 1.2 to 1.4 mm. in length. Sixty to ninety pairs of pre-anal papillae have been counted, the greater number of specimens having a larger number of these structures.

The tail of the male is relatively short, 0.18 to 0.22 mm. long. A narrow ala, continuous around the end of the tail is about 0.04 mm. in marginal extent. It meets lateral alae 0.02 to 0.03 mm. wide that terminate just anterior to the cloaca. Pre-cloacal papillae number 60 to 90 pairs reaching an anterior limit 5.0 mm. from the end of the body. Post-cloacal papillae

numbering six to eight pairs, differ in arrangement. Nearest the tip in some specimens three pairs occur closely in line parallel to the body margin; a fourth marginal pair is anterior to these and a fifth pair lies anteriorly toward the midline. Two pairs extend from below the lateral margins of the cloaca toward the alae and just at the corners of the cloaca two pairs are in close contact. The third of the three posterior pairs is not infrequently absent; in specimens showing this condition the papillae are arranged in well defined groups of two, of which the immediately post-colocal group sometimes includes a pair of double papillae toward the midline. No other differences may be noted in association with these variations in papillary arrangement.

In these helminths the male genital tract shows a noteworthy condition. The vas deferens, immediately after its origin, is folded in a longitudinal sigmoid curve from which it proceeds directly to the cloaca. Within a limited area at the beginning of the direct portion of the deferens the lining epithelium is decorated with hair-like processes. These occur in clusters of about 30 placed about 25μ apart in longitudinal rows. The rows, 35μ apart, are laterally staggered, so that the groups of processes alternate around the organ. The individual processes are relatively uniform in length, from 60 to 65μ ; 25 groups of these structures may be seen in cross section.

The material now available has all been preserved in formalin for a considerable time and it is not possible to determine whether these structures are, as their appearance strongly suggests, flagella. In describing "*Ascaris kuenthalii*" Cobb (5) noticed similar forms. His descriptions and illustrations prove that the structures he saw were identical with those in the present material. Apparently using living material, he reported motility for the processes and even attributed a definite function to them, that of assisting the movement of the spermatozoa on their way down the vas deferens. He says in part: "This" (the epithelium of the posterior part of the vas deferens) . . . "is provided with bundles of strong cilia which remind one of the not dissimilar elevations of the epithelium in *Ascaris lumbricoides*. In that case, however, the elevations are branching and show, according to Schneider and Lueckart, only amoeboid movement. Here, on the other hand, only bundles of often true cilia occur to which I must attribute a very active movement, not only because they show the composition of real cilia, but also, mainly because the free ends are directed posteriorly, and we find all the spermatozoa assembled in a central longitudinal bundle. In the space between the axial mass of sperms and the wall of the duct, i.e., in the space in which the cilia move, there are no spermatozoa."

Then follows a discussion of the grouping and extent of these structures, parallel in every way with those the author has noted. The observations of the author are further comparable in that the spermatozoa are invariably swept to the centre of the duct as Cobb has described.

Hetherington (6) in a discussion of ciliation quotes Cobb in an 1898 paper in which he discusses the motility of structures in the seminal vesicle without apparent reference to the worm in which he noted the condition; it would

seem that Cobb had in mind the observations quoted here and of which Hetherington was apparently unaware. The length of these processes, as well as their structural arrangement in distinct tufts, readily suggests their classification as flagella purely on the basis of appearance and arrangement. If, then, the evidence of Cobb's paper for their motility is accepted it must be concluded that this nematode species, at least, has flagella.

By far the greater bulk of our *D. leucas* material originated in the Gulf of St. Lawrence. Viscera of only three white whales have been available from other sources. Of these, one from Southampton Island, N.W.T., was parasite-free, one from Craig Harbour, Ellesmere Island, furnished a single immature *Anisakis* and the third, with origin at Lake Harbour, Baffin Island, was parasitized by a few immature *Anisakis* sp.

Acanthocephalids

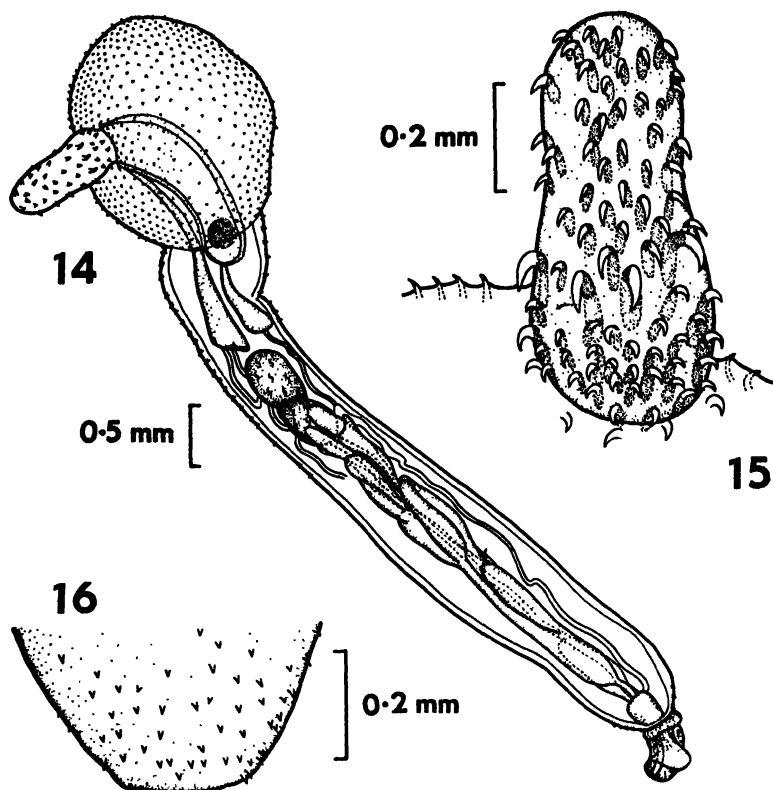
Corynosoma Luhe, 1911

Fixed firmly in the mucosa of the small intestine of seals, acanthocephalids of the species *C. strumosum* were collected from *Erignathus barbatus* taken at Cape Smith, Craig Harbour, Clyde River, and Pond Inlet, and from *Phoca hispida* at Cape Smith, Lake Harbour, Payne Bay, Stupart Bay, and Wolstenholme. *C. semerme* was found only once in a specimen of *E. barbatus* taken at Lake Harbour in August, 1939. In an earlier paper (4) an infection of *C. semerme* from dogs at Craig Harbour is recorded; though some of the seal acanthocephalid material from this post is very badly preserved there is little doubt that the specimens are *C. strumosum* rather than *C. semerme*. Though none of the Arctic material from *Delphinapterus leucus* included acanthocephalids, white whales in the Gulf of St. Lawrence were commonly parasitized by *C. strumosum*.

Corynosoma strumosum Rudolphi, 1892

Males are about 7 mm. long by 1.2 mm. wide in the region of the testes; females slightly larger when gravid. The body is straight, with almost parallel sides, up to an expansion at the anterior end where the body width is doubled. The proboscis is slightly barrel-shaped 0.85 mm. long by 0.35 mm. at its widest points. Spines in 18 rows decorate the organ; these are smallest at the base of the proboscis, extending about 0.04 mm. from the cuticle; at the fourth row they increase in size to 0.09 mm. then gradually decrease in size until, at the anterior tip, they are about 0.05 mm. The root of these spines is always about one-fourth greater than the free portion.

The proboscis narrows to the anterior third, then increases in size toward the base. The anterior expanded portion of the worm is thickly armed with small spines set deep in the cuticle; these increase in size at the base of the proboscis, and are continued into the body of the worm to a maximum point just below the posterior testis in the male and a corresponding position in the female. Spination is renewed terminally in the male. The proboscis sheath is relatively short, reaching a point just behind the testes; the lemnisci are truncated, not reaching the testes.



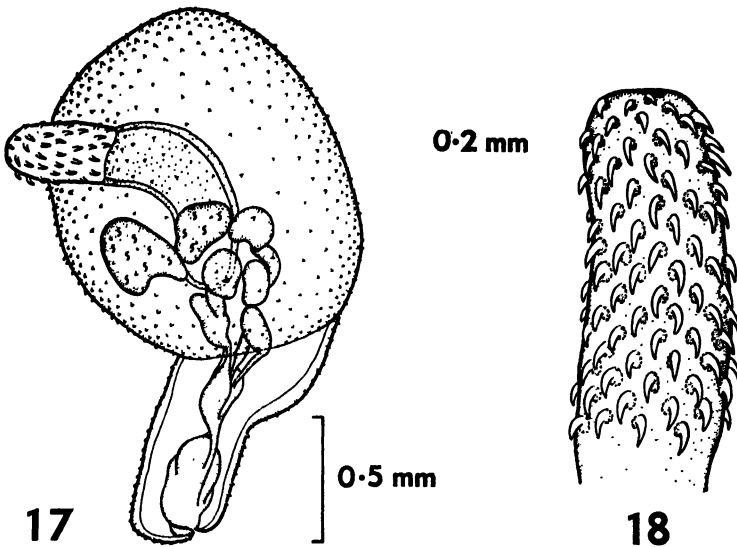
FIGS. 14 - 16. *Corynosoma strumosum* ex *E. barbatas*. 14. Entire worm, male. 15. Proboscis. 16. Detail of terminal spination, male.

The two testes of the male are roughly rectangular in shape, 0.5 by 0.3 mm., longitudinally colinear and in close contact with each other. The numerous cement glands form a compact mass running from the level of the middle of the posterior testes to the posterior end. Retinaculae follow an irregular course posteriorly from the brain. A conspicuous copulatory bursa is present. The female, apart from sexual structures, including the absence of terminal spines, is not dissimilar from the male. The body contains many embryos about 1.08 mm. by 0.5 mm. in size.

Corynosoma semerme Forssell, 1904

Males measure about 1.3 to 2.0 mm. by 0.9 to 1.0 mm.; females 2.8 to 3.0 mm. by 2.0 to 2.2 mm. The present specimens have been found to compare with the *C. semerme* material from dogs identified by Dr. Van Cleave. They have been assigned to this species on the criteria of size and shape and the specific arrangement of spines on the proboscis and body.

The short body, made up largely of an anterior expansion with a truncated rear-body is roughly comma-shaped. Spines in both sexes are continued into the rear quarter of the body dorsally and terminally on the lower side; in the male and female the end of the body is spined. The proboscis is armed with



FIGS. 17 - 18. *Corynosoma semerme* ex. *E. barbatus*. 17. Entire worm, male. 18. Detail of proboscis.

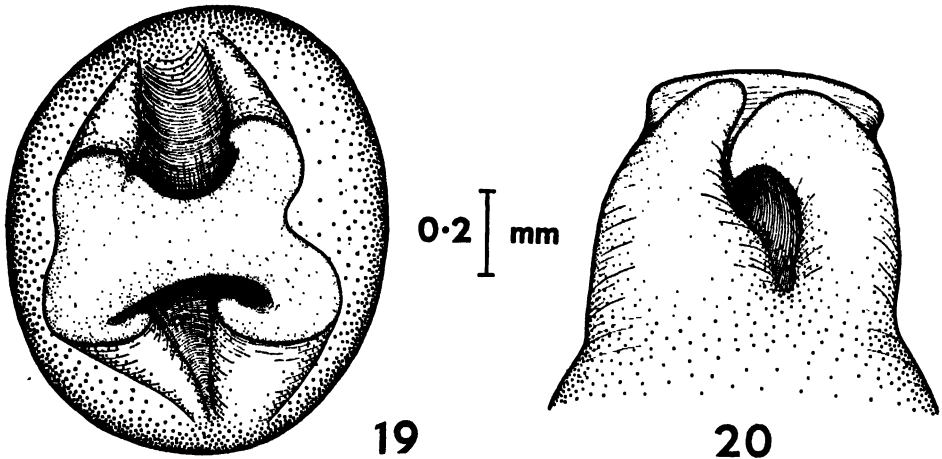
19 rows of spines. These are smallest in the first two rows (0.025 mm.), the remainder being uniform in size (0.062 mm.). The roots of these spines are never greater than about half the length of the free portion. The proboscis itself is distinctly more uniform in diameter than is the case with *C. strumosum* and measures about 0.7 by 0.25 mm. Organs are essentially similar to those of *C. strumosum* but more compactly arranged. The testes are laterally colinear, the lemnisci and proboscis sheath lie dorsally, and extend somewhat posterior, to them.

Cestodes

Cestodes require more careful technique in preserving and fixing than collections of this kind provide. For this reason, although considerable material has come to hand, all of it is in too poor condition to permit with certainty diagnosis, though it is identifiable beyond doubt as falling within the genus *Diphyllobothrium*. All specimens are much contracted and in most decomposition has occurred to a lesser or greater extent. The collection is made up largely of broken strobila, some with the scolices included. Several specimens are complete animals; these are made up of more than 75 segments and in their contracted condition measure about 25 by 6.0 mm. The eggs in balsam mounts measure about 0.053 by 0.035 mm.; there was no significant variation from this size.

In most cases three to four uterine loops may be noted but occasionally there are as many as seven. In all cases the uterus lies definitely behind the cirrus sac, the uterine pore lying ventral to about the left rear margin of the sac.

The bothria are deep, separated by a frontal ridge, but reaching the anterior limit of the scolex in lateral perspective and may be traced to the posterior



FIGS. 19 - 20. *Diphyllobothrium* sp. ex *E. barbatus*. 19. En face view. 20. Scolex, ventral view.

margin of the scolex. The scolex overlies the anterior part of the first segment behind. In heavily contracted specimens the scolex was roughly triangular in shape; isolated scolices were rectangular.

The differences in shape of the scolex and in the number of uterine coils would, by some standards, require species distinction. Stunkard and Schoenborn (12) have included these characteristics under *D. lanceolatum* and its synonyms, *D. schistochilos* and *D. coniceps*. Until it is possible to examine fresh material a diagnosis of this material as *D. lanceolatum* is indicated, and all distinguishable details conform.

The measurements do not disagree with the range listed for *D. lanceolatum*. The testes occupy a single medullary layer. The cirrus sac has a maximum diameter of 0.12 mm. with a relatively long, usually protruded cirrus; the double ovary has entire margins.

Further accurate details are not distinguishable.

The proglottids are longer than wide (1.8 by 2.4 mm. or wider than long (3.6 by 1.6 mm.). In a complete and highly compressed animal the proglottids were widest at about half the total length and were 0.4 mm. long and 5.5 mm. wide; the subterminal segment was 0.6 mm. long and 1.0 mm. wide.

Cestodes of this genus were recovered in *Erignathus barbatus* from Cape Smith, N.W.T., Cape Dorset, Baffin Island, and Wolstenholme, Que., and in *Phoca groenlandica* from Lake Harbour and Clyde River, Baffin Island.

The following host-parasite list summarizes our definite records in Canada to date:

Delphinapterus leucas (White Whale)

Anisakis simplex

Anisakis sp. inq.

Corynosoma sirumosum

*Erignathus barbatus**Porrocaecum decipiens**Corynosoma semerme**C. strumosum**Diphyllobothrium* sp.*Phoca vitulina* (Harbour or Jar Seal)*Contracaecum osculatum**Phocascaris netsiki**Phoca groenlandica* (Harp or Greenland Seal)*Porrocaecum decipiens**Diphyllobothrium* sp.*Phoca hispida* (Ringed Seal)*Contracaecum osculatum**Corynosoma strumosum**Phocascaris netsiki*

Of these, three records are new, the occurrence of *C. strumosum* in *D. leucas*, of *P. netsiki* in *P. hispida* and of *C. semerme* in *E. barbatus*.

Acknowledgments

The active co-operation of the Commissioner and men of the Royal Canadian Mounted Police, of the Fur Trade Commissioner and traders of the Hudson's Bay Company and of Dr. Vladykov and his colleagues was invaluable in collecting the material upon which this paper is based. The author is also keenly appreciative of the privileges extended by the Administration of the Northwest Territories associated with his appointment to the 1939 Eastern Arctic Patrol, which allowed personal collecting in Ellesmere, Baffin, Somerset and Southampton Islands and the western coast of Hudson Bay from the R.M.S. "Nascopie".

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THE DISTRIBUTION OF THE RED AND YELLOW COLOURS OF THE MUSCLE TISSUE OF BRITISH COLUMBIA CANNED SPRING SALMON AROUND THE MEANS OF THE INDIVIDUAL DISTRIBUTIONS OCCURRING IN SMALL, ARBITRARILY CHOSEN TIME INTERVALS¹

BY F. CHARNLEY² AND LAURA M. HARCUS³

Abstract

The distributions of the red and yellow colours of the cooked muscle tissue of British Columbia spring salmon (*Oncorhynchus Tshawytscha*) around the means of the individual distributions occurring in small, arbitrarily chosen time intervals are composite distributions each consisting of two component normal distributions, thus indicating that there are two, and only two, varieties of this species when the salmon are classified on the basis of these two quality characteristics. The proportions of the pale and red varieties are very nearly 1 : 3 so that when sampling fluctuations are taken into account the data are in complete agreement with the hypothesis that the true proportions are, respectively, $\frac{1}{4}$ and $\frac{3}{4}$.

In an investigation of the functional forms of the distributions of the red and yellow colours of the muscle tissue of British Columbia canned salmon in connection with the grading of these characteristics, it was found that the distribution of the red colour of canned spring salmon (*Oncorhynchus Tshawytscha*) is markedly bimodal. The general shape of the distribution suggested that it was a composite distribution consisting of two component normal distributions with the red component forming considerably more than half the total population. If this hypothesis is correct and the species is homogeneous biologically, the data would indicate that the pale and red colours in this case are alternative Mendelian characters.

Before an answer to this last question can be undertaken, however, it is necessary to show that the distribution of the red colour in this species is in fact a mixture of two populations. Accordingly, the object of the investigation reported in this paper was to test the hypothesis as regards the functional form of the red colour and also to investigate that of the yellow colour of this species by fitting the experimental data with composite distributions made up of two component normal distributions.

Selection and Tabulation of Data

The data employed in the investigation were taken from the records of routine examinations of British Columbia canned spring salmon for the 1938 season and consist of the colour measurements made during these

¹ Manuscript received June 3, 1940.

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³ Statistical Assistant.

examinations. The measurements were carried out by means of an Armstrong Colorimeter, an instrument (1) that utilizes the principle of matching the colour of the sample against coloured glasses. The intensity of colour is accordingly expressed in red and yellow Lovibond units.

In tabulating the original data preparatory to investigating the distributions of the red and yellow colours of the cooked muscle tissue of this species it was necessary to eliminate, as far as possible, three types of variations in the results. These were: (i) Variations arising from the fact that a number of companies pack only the red variety of this species, or, at least, only those salmon which, as far as can be determined, will definitely show red muscle tissue when cooked. (ii) Variations arising through geographical effects. For example, sockeye salmon caught in the northern areas in British Columbia are somewhat less intensely coloured than salmon caught in the southern areas, or in the Fraser River. The same seems to be true also of spring salmon. (iii) Variations resulting from seasonal effects.

Variations due to the first of these factors were eliminated by making careful inquiries of the companies who, as a result of preliminary inquiries, were believed to pack a certain proportion of, or all, unselected spring salmon. This information gave the canneries, in the area chosen, that had packed only unselected spring salmon during the 1938 season.

Variations resulting from the second factor were eliminated, as far as possible, by restricting the samples included in the investigation to those from the canneries in the area north of Knight Inlet that packed unselected samples during the 1938 season.

TABLE I

DISTRIBUTIONS OF THE RED COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

Red colour, Lovibond units	Interval No.																
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1.5	1	1	2	2	1			1	2	1							2
2.0	5	1	1	7	5	4	2	1	2	3	3	3	4	4	1		2
2.5		2	2	1	2	1	1	1	2	2	5	1	1	2	1		4
3.0	2	1	2				1	2		4	5	1	3	2	1	4	3
3.5	4	1			2	1	2	1	1	2	3	2	4	1	2		2
4.0	6	2	5	4	3	1		4	2	2	6	3	1	2	2		4
4.5	8	2	6	9	1	3	8	2	4	13	12	2	5	2		2	2
5.0	4	4	6	2	4	3	7	6	6	5	10	5	4	3			
5.5	3		1	5	1	3	1	2	2	10	1	2	2	1			3
6.0	1		1	2		1		2	3						1		
6.5										1	1						1
7.0										1							
7.5										1							
Total	34	14	26	32	19	17	22	22	24	46	46	20	24	18	7	6	23

TABLE II

DISTRIBUTIONS OF THE YELLOW COLOUR OF THE MUSCLE TISSUE IN UNSELECTED SAMPLES OF BRITISH COLUMBIA CANNED SPRING SALMON PACKED IN CERTAIN CANNERIES IN THE NORTHERN AREA DURING 1938

Yellow colour, Lovibond units	Interval No.																		
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
1.5	2	1				1			1										
2.0	3	1	1	3	3	1	3	1	2	2	1	1	2	3		1	3		
2.5	6	2	6	8	5	5	2	4	4	6	13	5	10	6	4	1	9		
3.0	12	5	8	9	3	1	6	8	7	17	10	4	5	6	2	4	5		
3.5	8	2	4	5	2	6	7	4	5	11	10	4	4	1	1		3		
4.0	1	2	2	5	5	1	2	4	3	5	12	5	2	2			2		
4.5	2	1	5	2	1	2	2	1	2	3		1	1				1		
5.0										2									
Total	34	14	26	32	19	17	22	22	24	46	46	20	24	18	7	6	23		

Elimination of the variations arising from the third factor, however, was somewhat more difficult, because the mean intensities of the red and yellow colours of the muscle tissue of canned spring salmon show considerable seasonal trend. Since the distribution required is the distribution of the red or yellow colour around the line of seasonal trend and the absolute values of the means were of no particular interest, a simple method of combining the data evidently was to adjust the individual distributions occurring in small arbitrarily chosen time intervals to some convenient mean also arbitrarily chosen. For convenience in tabulating, the data were grouped in five-day intervals, the samples in any one time-interval thus being assumed to be derived from salmon of substantially the same biological condition. Time intervals were in all cases determined by the code marks.

Tables I and II show the distributions of the red and yellow colours of the samples of canned spring salmon when the samples are grouped in five-day intervals, while the trends in the means of the distributions given in Tables I and II are illustrated graphically in Fig. 1. From an inspection of the trends

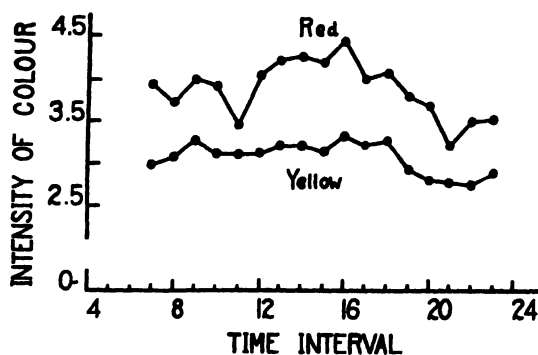


FIG. 1. Curves showing seasonal trends in means of red and yellow colours of the muscle tissue of canned spring salmon corresponding to the data of Tables I and II.

in Fig. 1 it will be observed that it is not necessary in all cases to shift the individual distributions to the arbitrary mean, since the distributions can be readily arranged into groups of approximately the same mean intensity of colour. A number of the means of the red colour lie on, or close to, the intensity 4.2. For convenience this was accordingly chosen as the arbitrary mean of the red colour.

To shift the remaining groups to this arbitrary mean we proceed as follows: Let h = grouping interval, d = shift in mean = $m_0 - m_i$, where m_i is the mean of the distribution that it is required to shift to the mean m_0 . Then for $d < h$ the proportion of frequencies remaining in any one interval is $\frac{h-d}{h}$. The proportion of frequencies in the interval that are to be moved to the next or some succeeding interval is therefore $\frac{1-h-d}{h} = \frac{d}{h}$. These formulae evidently apply also when the absolute value of d is greater than h , since, when the distribution is moved an integral number of intervals, its frequencies remain unchanged. To check the calculation the mean of the new distribution is calculated and compared with the arbitrary mean. An illustration of the calculations is shown in Table III.

TABLE III

DETAILS OF CALCULATIONS FOR INTERVAL No. 7 OF TABLE I ILLUSTRATING METHOD OF SHIFTING MEANS OF INDIVIDUAL DISTRIBUTIONS TO ARBITRARY MEAN.

X = intensity of red colour; f = frequency in original distribution; f_{i+1} = frequencies moved to next interval; f_i = frequencies remaining in interval; F = frequency in new distribution.

X	f	f_{i+1}	f_i	F	x	Fx
1.5	1	—	0.48	0.48	-5	- 2.40
2.0	5	0.52	2.40	2.92	-4	-11.68
2.5	—	2.60	—	2.60	-3	- 7.80
3.0	2	—	0.96	0.96	-2	- 1.92
3.5	4	1.04	1.92	2.96	-1	- 2.96
4.0	6	2.08	2.88	4.96	0	—
4.5	8	3.12	3.84	6.96	1	6.96
5.0	4	4.16	1.92	6.08	2	12.16
5.5	3	2.08	1.44	3.52	3	10.56
6.0	1	1.56	0.48	2.04	4	8.16
6.5	—	0.52	—	0.52	5	2.60
Total	34			34.00		13.68

$d = 0.26; h - d = 0.24$. Distance from arbitrary origin = 0.402. Mean = 4.201.

In combining the data of Table I the distributions in intervals No. 13, 14, and 15 were assumed to have the same mean, namely 4.2, while the pairs of intervals 9 and 10, 17 and 18, and 22 and 23 have approximately the means 3.97, 4.04, and 3.51 respectively. Similarly in the case of the yellow colour the mean of each of the intervals 13 and 14, namely 3.2, was taken as the arbitrary origin, while intervals 10, 11, 12, and 15 were assumed to have the same mean 3.14, intervals 16, 17, and 18, the mean 3.25, and intervals 20, 21, and 22, a mean of 2.78.

Method of Fitting Combined Distributions

Table IV shows the combined or total distributions of the red and yellow colours derived from Tables I and II by combining the distributions occurring in the individual intervals as described in the preceding paragraphs. The general appearance of the distributions of the red and yellow colours of the muscle tissue of canned spring salmon when fewer intervals are combined is shown graphically in Figs. 2 and 3. In all instances, it will be observed, there is evidence that the distribution of the red colour is bimodal. The distribution of the yellow colour, however, is apparently unimodal. In the combined distribution of the red colour given in Table IV the dispersion of the measures around the two modes suggests that the distribution is a mixture of two component normal distributions. Since one of the components is present in smaller quantity than the other and very probably differs in standard deviation from the other, the data correspond to the general case of two component normal distributions.

TABLE IV

COMBINED DISTRIBUTIONS OF RED AND YELLOW COLOURS OF CANNED SPRING SALMON DERIVED FROM THE DATA OF TABLES I AND II

X = colour, F = frequency

Red colour				Yellow colour	
X	F	X	F	X	F
1.0	0.48	5.0	78.28	1.5	4.16
1.5	7.64	5.5	45.94	2.0	25.86
2.0	27.02	6.0	22.66	2.5	79.86
2.5	36.20	6.5	5.30	3.0	110.66
3.0	28.80	7.0	2.94	3.5	89.72
3.5	29.96	7.5	0.90	4.0	57.70
4.0	43.22			4.5	26.72
4.5	70.66			5.0	5.32
Total			400		400

The equations required in solving for the parameters of the component normal distributions in this case are readily derived from the characteristic function (2), namely,

$$\phi(t) = l_1 e^{a_1 t + b_1^2 t^2 / 2} + l_2 e^{a_2 t + b_2^2 t^2 / 2},$$

by taking successive derivatives of this function with respect to t . On putting $t = 0$ in the results we easily obtain the successive moments of the distribution in terms of the unknown proportions l_1 and l_2 , the means a_1 and a_2 , and the standard deviations b_1 and b_2 of the component normal distributions. The required moments are as follows:

- (1) $\mu_0 = 1 = l_1 + l_2$
- (2) $\mu_1 = l_1 a_1 + l_2 a_2$
- (3) $\mu_2 = l_1(a_1^2 + b_1^2) + l_2(a_2^2 + b_2^2)$
- (4) $\mu_3 = l_1 a_1^3 + 3l_1 a_1 b_1^2 + l_2 a_2^3 + 3l_2 a_2 b_2^2$

$$(5) \quad \mu_4 = l_1(a_1^4 + 6a_1^2b_1^2 + 3b_1^4) + l_2(a_2^4 + 6a_2^2b_2^2 + 3b_2^4)$$

$$(6) \quad \mu_5 = l_1(15b_1^4a_1 + 10b_1^2a_1^3 + a_1^5) + l_2(15b_2^4a_2 + 10b_2^2a_2^3 + a_2^5),$$

in which μ_0, μ_1 , etc. are the various moments of the combined distribution.

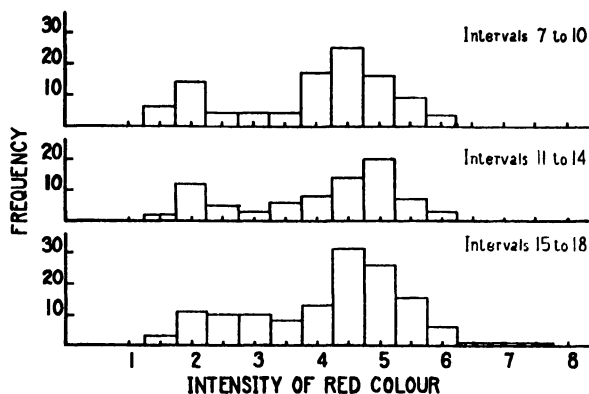


FIG. 2. Distributions of intensity of the red colour obtained on combining the individual distributions in four successive time intervals.

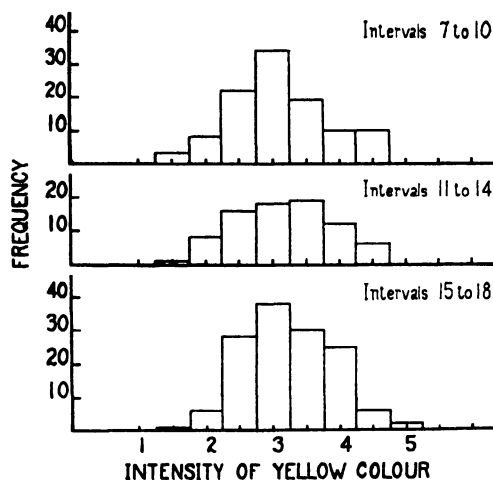


FIG. 3. Distributions of intensity of the yellow colour obtained on combining the individual distributions in four successive time intervals.

These equations, which were first derived by Pearson, permit a solution of the general case of a distribution consisting of two component normal distributions. An examination of the combined distribution of the red colour given in Table IV, however, shows that at least half, and possibly more than half, of the distribution in the upper part of the range, that is, a large portion of the red variety in the distribution, may be considered homogeneous. Hence, instead of solving Equations (1) to (6) by the laborious procedure of elimination and solution of the resulting nonic, we may employ the relatively rapid method described by Pearson (5).

Let d be the distance of the stump of the tail from the mean of the truncated portion and Σ the standard deviation of the truncated portion about its mean. Also let ν_1^1 , ν_2^1 be the first and second moments of the truncated portion around an arbitrary origin x_0 units distant from the stump, $\mu_2 = \sigma^2$ the second moment of the normal distribution around its mean, x the distance from the stump to the mean of the curve, \bar{x} the distance of the mean of the truncated portion to the mean of the curve, n the area of the truncated portion, N the whole population, m_0 , m_1 , etc., the various normal moment functions, and i the grouping interval. Then as shown by Pearson

$$d = x_0 + \nu_1^1 i = \bar{x} + x$$

$$\Sigma^2 = \nu_2^1 i^2 = \{\nu_2^1 - (\nu_1^1)^2\} i^2$$

$$(7) \quad \frac{n}{N} = \frac{1}{2} + m_0$$

$$(8) \quad n\bar{x} = N\sigma \left(\frac{1}{\sqrt{2\pi}} - m_1 \right)$$

$$(9) \quad n\mu_2 = N\sigma^2 \left(\frac{1}{2} + m_2 \right)$$

$$(10) \quad \frac{d}{\sigma} = \frac{\frac{1}{\sqrt{2\pi}} - m_1 + \frac{x}{\sigma} \left(\frac{1}{2} + m_0 \right)}{\frac{1}{2} + m_0}$$

$$(11) \quad \frac{\Sigma^2}{d^2} = \frac{\left(\frac{1}{2} + m_2 \right) \left(\frac{1}{2} + m_0 \right) - \left(\frac{1}{\sqrt{2\pi}} - m_1 \right)^2}{\left\{ \frac{1}{\sqrt{2\pi}} - m_1 + \frac{x}{\sigma} \left(\frac{1}{2} + m_0 \right) \right\}^2}$$

The graph of the combined distribution of the red colour given in Table IV suggests that it is safe to assume the truncated portion bounded by the stump 3.75 red units as being very closely homogeneous. Accordingly, the first and second raw moments ν_1^1 and ν_2^1 (Table V) are respectively 2.8752 and 10.0940, n is 269.90, Σ , 1.8272 and d , 1.1876. Hence $\frac{\Sigma^2}{d^2} = \psi_1 = 0.3239$. By trial it is found on applying Equation (11) that this value of ψ_1 corresponds

TABLE V

STATISTICAL DATA USED IN CALCULATING THE MEANS AND STANDARD DEVIATIONS OF THE COMPONENT DISTRIBUTIONS IN THE COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS

Distribution of red colour

Arbitrary origin = 4.0; $\mu_1^1 = 0.4020$ half units; $\mu_2 = 6.2229$ half units²; $\mu_1 = 0$; $\mu_2 = 1.5557$ units; Total number of measures = 400.

Truncated portion of distribution of red colour

Arbitrary origin = 3.5; stump = 3.75 red units; $n = 269.90$; $\nu_1^1 = 2.8752$ half units; $\nu_2 = 10.0940$ half units²; $d = 1.1876$; $\Sigma^2 = 0.4568$ units.

Distribution of yellow colour

Arbitrary origin = 3.0; $\mu_1^1 = 0.4063$ half units; $\mu_2 = 2.0021$ half units²; $\mu_1 = 0.5283$ half units²; $\mu_2 = 10.5560$ half units⁴.

to a value of $x' \left(= \frac{x}{\sigma} \right)$ lying between 1.2 and 1.3. On calculating the value of ψ_1 corresponding to $x' = 1.4$ and applying Stirling's interpolation formula or Formula (iii) in Pearson's "Tables for Statisticians and Biometricians" to the three values 1.2, 1.3, and 1.4 of x' and the corresponding values of ψ_1 , we find that x' corresponding to the above value of ψ_1 , namely 0.3239, is 1.2965. Application of the first two terms of the interpolation formula to the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the same three values of x' then gives the values of $\frac{1}{2} + m_0$ and $\frac{1}{\sqrt{2\pi}} - m_1$ corresponding to the calculated value of x' , that is, to $\psi_1 = 0.3239$. The substitution of these values together with the experimental value of d in Equation (10) gives $\frac{d}{\sigma} = 1.4872$, and hence $\sigma = b_1 = 0.7985$. Consequently $x = x'\sigma = 1.0353$; $a_1 = 3.75 + 1.0353 - 4.201 = 0.5843$; $\frac{n}{N} = \frac{1}{2} + m_0 = 0.9026$, and $N = 299.027$.

To find the mean and standard deviation of the other component distribution, that is, of the pale variety of spring salmon, we substitute the parameters of the red component in Equations (1), (2), and (3). The value of l_1 is 0.7476, so that l_2 is 0.2524. The first moment of the combined distribution μ_1 is zero and the second moment $\mu_2 = 1.557$. Equations (2) and (3) therefore give $a_2 = -1.7304$ and $b_2 = 0.5188$ or, referred to the arbitrary mean, $a_2 = 2.4706$.

As mentioned above, the combined distribution of the yellow colour is uni-modal and from an examination of the plotted data appears to be approximately normal. The values of the statistics k and β_2 of this distribution, however, suggest that this distribution also has been drawn from a non-normal population. The values of k and β_2 are 0.1865 and 2.6334, respectively. Assuming that the distribution of the yellow colour is approximately normal we have $\sigma_k = \sqrt{\frac{6}{n}} = 0.1225$ and $\sigma_{\beta_2} = \sqrt{\frac{24}{n}} = 0.2450$,

so that the calculated values deviate approximately 1.5 standard deviations from the expected values in each case. The values of k and β_2 , however, also deviate in the directions that would be expected if the combined distribution of the yellow colour consists of two component distributions similar to those in the combined distribution of the red colour but differing much less in their means. The chances of drawing two simultaneous values of k and β_2 from a normal population each deviating 1.5 standard deviations from the expected value is about 1 chance in 60 and the probability that the two values will deviate in this particular direction is one-quarter of this. Hence there is a strong indication that the distribution of the yellow colour is also a mixture of two component populations corresponding to those in the distribution of the red colour.

In view of the close chemical relationship that exists between chlorophyll and the blood pigments and also the close biological relationship that exists

between the red and yellow colours of canned salmon as indicated by the relatively high correlation between the red and yellow colours, it seems safe to assume that the proportions l_1 and l_2 of the two varieties in the combined distribution of the yellow colour will be the same as those found for the distribution of the red colour. With this assumption the parameters of the component distributions of the yellow colour may then be calculated by means of Equations (2) to (5) from the first four moments of the distribution. The required moments are $\mu_1 = 0$, $\mu_2 = 2.0021$, $\mu_3 = 0.5283$ and $\mu_4 = 10.5560$. Substitution of these values of the moments and the values of l_1 and l_2 , namely 0.7476 and 0.2524 respectively, in Equations (2) to (5) thus gives four equations in the unknown a_1 , a_2 , b_1 , and b_2 , from which by the elimination of a_2 , b_1 , and b_2 we obtain the sextic $a_1^6 + 0.05497a_1^3 - 0.05845a_1^2 - 0.00125 = 0$.

The application of Descartes' rule of signs shows immediately that this equation has only one real positive root. Putting $a_1 = 0$ and $a_1 = 1$ in this equation we find that $F(0) = -0.002036$ and $F(1) = 1.0032$ and hence the root lies between 0 and 1. By applying Horner's method the positive root is then found to be $a_1 = 0.4474$.

Since the mean of the combined distribution measured from the arbitrary origin 3.0 was in terms of half the original units as were also the moments, the resulting values of the parameters are also in half units. The mean of the red variety in half units was 0.4062. Accordingly, the mean of the red variety is $3.2031 + 0.2237 = 3.4268$ yellow units. The value of a_2 follows at once and is $a_2 = -1.2552$, that is, the mean of the pale variety is 2.5755 yellow units. Similarly, the corresponding standard deviations are $b_1 = 1.2985$, $b_2 = 0.8771$, so that when expressed in whole units the standard deviations of the yellow colours of the red and pale varieties are 0.6493 and 0.4385, respectively.

Test of Hypothesis

To test the hypothesis as regards the functional forms of the two combined distributions we compare the theoretical distributions with the experimental distributions given in Table IV. This comparison may be effected either graphically or by means of the χ^2 test or by both methods.

Table VI shows the calculated or theoretical values of the ordinates corresponding to given values of the intensity of colour for each of the experimental distributions given in Table IV. These data are illustrated graphically in Figs. 4 and 5. From an inspection of these figures it will be observed that the theoretical distributions describe with a high degree of accuracy the experimental data.

This conclusion is also confirmed by the χ^2 test. Tables VII and VIII show the observed and theoretical frequencies falling into the various classes in the combined distributions of the red and yellow colours. The number of classes and the value of χ^2 for the combined distribution of the red colour are respectively 11 and 2.080, and the corresponding figures for the combined

TABLE VI

ORDINATES OF THE COMPONENT NORMAL DISTRIBUTIONS AND OF THE RESULTING COMBINED DISTRIBUTIONS OF THE RED AND YELLOW COLOURS CORRESPONDING TO THE DATA OF TABLE IV

Colour	Red			Yellow		
	Pale variety	Red variety	Combined	Pale variety	Red variety	Combined
1.0	0.72		0.72	0.07	0.09	0.16
1.5	6.77		6.77	2.30	1.17	3.47
2.0	26.08	0.19	26.27	19.54	8.48	28.02
2.5	38.79	1.25	40.04	45.29	33.88	79.17
3.0	23.54	6.29	29.83	29.20	74.73	103.93
3.5	5.51	21.08	26.59	5.16	91.34	96.50
4.0	0.52	46.34	46.86	0.25	62.53	62.78
4.5		70.41	70.41		23.65	23.65
5.0		72.38	72.38		4.95	4.95
5.5		50.51	50.51		0.58	0.58
6.0		23.59	23.59			
6.5		7.69	7.69			
7.0		1.63	1.63			
7.5		0.24	0.24			

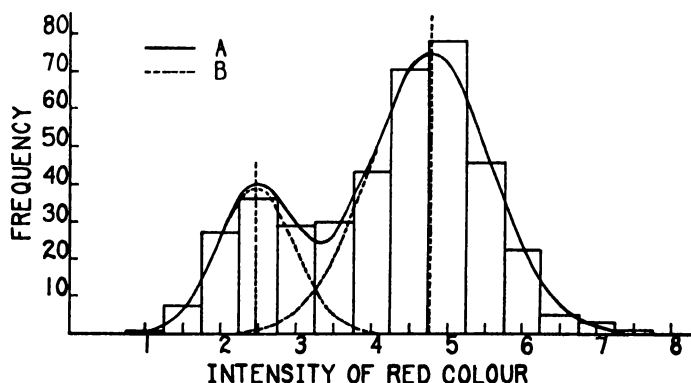


FIG. 4. Graphical illustration of the combined distribution of the intensity of the red colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.

distribution of the yellow colour are 8 and 2.257. Since the observed data in this case were used in calculating the values of the parameters in the theoretical distribution, the number of classes (7) must be diminished by six in each case in computing the number of degrees of freedom, since this is the number of conditions employed in calculating the values of the parameters. The number of degrees of freedom of the distributions of the red and yellow colours are accordingly 5 and 2 respectively. Reference to Fisher's table (3) shows that both these values of χ^2 might very easily have arisen through sampling fluctuation. The probability associated with the value 2.080 is approximately $P = 0.85$, while that associated with the value

2.257 is about $P = 0.50$. There is thus no reason to suspect the hypothesis tested and the theoretical distribution must, therefore, be regarded as fitting very closely the experimental data.

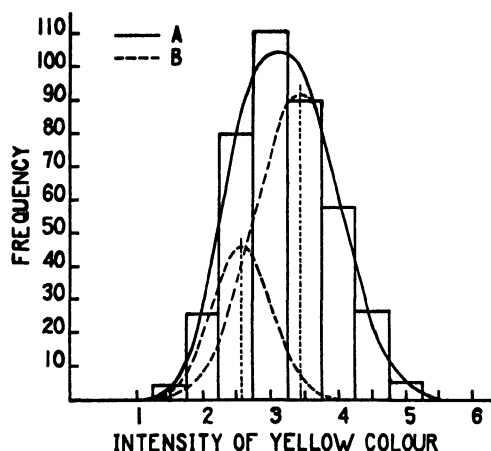


FIG. 5. Graphical illustration of the combined distribution of the intensity of the yellow colour showing experimental and theoretical distributions. A = combined distribution; B = component distributions.

TABLE VII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE RED COLOUR

Colour	Obs'd f ($m + x$)	Calc'd f (m)	x	$\frac{x^2}{m}$
0.5 } 1.0 } 1.5 }	8.12	8.32	-0.20	0.005
2.0 }	27.02	25.73	1.29	0.065
2.5 }	36.20	38.74	-2.54	0.166
3.0 }	28.80	29.62	-0.82	0.023
3.5 }	29.96	27.00	2.96	0.324
4.0 }	43.22	46.68	-3.46	0.256
4.5 }	70.66	69.12	1.54	0.034
5.0 }	78.28	70.96	7.32	0.755
5.5 }	45.94	49.88	-3.94	0.311
6.0 }	22.66	23.98	-1.32	0.073
6.5 } 7.0 } 7.5 } 8.0 }	9.14	9.96	-0.82	0.068
Total	400.00	399.99		2.080

Concluding Remarks

Sheppard's corrections for grouping, it will be observed, were not applied in the foregoing calculations, but instead, the raw moments were substituted directly into the equations. Shewhart (7) gives a number of reasons why it may not be advisable in all cases to apply such corrections. In the present

TABLE VIII

COMPARISON OF OBSERVED AND THEORETICAL FREQUENCIES IN THE COMBINED DISTRIBUTION OF THE YELLOW COLOUR

Colour	Obs'd f ($m + x$)	Calc'd f (m)	x	$\frac{x^2}{m}$
1.0 } 1.5 }	4.16	4.47	-0.31	0.021
2.0	25.86	29.08	-3.22	0.356
2.5	79.86	76.97	2.89	0.108
3.0	110.66	101.61	9.05	0.806
3.5	89.72	95.00	-5.28	0.293
4.0	57.70	62.24	-4.54	0.331
4.5	26.72	24.42	2.30	0.217
5.0 } 5.5 }	5.32	6.20	-0.88	0.125
6.0 }				
Total	400.00	399.99		2.257

instance the most cogent reason for not applying the corrections would appear to be the relatively small sample size (269.9) employed in calculating the second moment of the truncated portion of the distribution of the red colour. If h is the grouping interval, Sheppard's correction for the second moment is $-\frac{h^2}{12}$. In this instance, however, the standard deviation of the second moment is (4, 6).

$$\sigma_{\mu_2} = \sqrt{\frac{\mu_4 - \mu_2^2}{n}},$$

where n is the sample size. The values of μ_4 and μ_2 of the truncated part expressed in half units were 11.4430 and 1.8272 respectively. Hence, in half units $\sigma_{\mu_2} = 0.1733$ or $\sigma_{\mu_2} = 0.04332$ red units. Sheppard's correction, namely, 0.02083, is thus less than half the standard error of the second moment, so that it would be a useless refinement to apply the correction in this case.

Another point that should be noted in the above calculations is the approximation employed in combining the individual distributions in the various time intervals. The means of the individual distributions have been used as estimates of the true means of the red and yellow colours corresponding to the respective time intervals. This procedure will introduce slight errors into the final results owing to sampling fluctuations in the means. Here again, however, the simpler procedure would seem to be preferable in this case to the questionable refinement of fitting the trends with meaningless third or fourth degree power series.

Finally, it is interesting to note that the difference in the means of the red colour for the two varieties is $4.7853 - 2.4706 = 2.3147$ and the difference in the means of the yellow colour, $3.4268 - 2.5755 = 0.8513$. The absolute values of the means, of course, are arbitrary. Also, the two varieties of spring salmon occur very nearly in the proportions of 1 to 3, that is, very nearly one-

quarter of all the samples consist of the pale variety while the remaining three-quarters is made up of the red variety of this species.

The value 0.7476, it will be observed, is in complete accord with the hypothesis that the true proportion is 0.7500. For, if a sample of 400 were drawn from a population in which the true proportion of the red variety was 0.7500, the standard deviation of the observed proportion would be

$$\sigma_p = \sqrt{\frac{pq}{n}} = 0.02165.$$

Consequently, the observed value 0.7476 differs only slightly more than one-tenth standard deviation from the hypothetical value 0.7500 and there are accordingly no grounds for concluding that the true proportion is not 0.7500.

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PARASITES OF FRESHWATER FISH

III. FURTHER STUDIES ON THE INTERNAL TREMATODES OF FISH IN THE CENTRAL ST. LAWRENCE WATERSHED¹

By M. J. MILLER²

Abstract

Three new species of internal trematodes, *Plagioporus serratus* sp. nov., *Phyllodistomum lysteri* sp. nov., and *Parastiotrema ollawanensis* gen. et sp. nov., are described from Canadian freshwater fish. *Plagioporus serotinus* Stafford, 1904, is redescribed, and the genus *Caudotestis* Yamaguti, 1934, is reduced to synonymy with *Plagioporus* Stafford, 1904. *Anallocreadium pearsei* Hunter and Bangham, 1932, is considered synonymous with *A. armatum* McCallum, 1895. The genus *Bunoderina* Miller, 1936, is reduced to synonymy with *Bunodera* Railliet, 1896.

Introduction

This paper, the third in a series on the parasites of freshwater fish in Canada, presents the results of the survey that has been continued since the first paper (6) appeared; it deals with non-commercial as well as commercial fish. All the fish were taken from the Ottawa River near its confluence with the St. Lawrence River, at Ste. Anne de Bellevue, Quebec.

Species Recovered

FAMILY ALLOCREADIIDAE

Genus *Plagioporus* Stafford, 1904

Synonyms: *Lebouria* Nicoll, 1909,

Caudotestis Yamaguti, 1934.

Stafford created the genus *Plagioporus* to include *P. serotinus*, a trematode he recovered from the intestines of *Moxostomata macrolepidotum* (red horse sucker). Nicoll (9) erected the genus *Lebouria* for his species *L. idonea* and included in the genus a trematode described by Linton from *Bairdiella chrysura* and named by Nicoll *L. abducta*. Price (12) pointed out that *Lebouria* was congeneric with *Plagioporus* and, as the latter genus has priority, he transferred the species of *Lebouria* to the genus *Plagioporus*, thus reducing *Lebouria* to synonymy. The subgenus *Caudotestis* (Issaitschikow, 1928) was raised to generic rank by Yamaguti (16) to include those species of *Plagioporus* having the intestinal caeca terminating at the level of the testes, and the vitellaria usually not extending into the post-testicular body region. However, these two characters are not sufficiently well defined in the species of the two genera, which show all the intergradations. Thus, *P. cooperi* has the caeca terminating at the level of the testes, but the post-testicular vitellaria

¹ Manuscript received June 27, 1940.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada, awarded through the National Committee on Fish Culture.

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subspherical, and measures from 0.08 to 0.14 mm. in diameter. The acetabulum is situated two-fifths of the body length from the anterior tip, and is approximately twice the diameter of the oral sucker. There is a short pre-pharynx and a well developed pharynx whose length is about half the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The intestinal caeca extend to within a short distance of the posterior extremity. The testes are in tandem arrangement, one immediately behind the other, in the middle of the posterior half of the body. Occasionally the anterior testis is displaced slightly to the left. The testes are smooth-margined and usually somewhat flattened in the antero-posterior axis. The ovary is spherical and is situated immediately anterior of the anterior testis, on the right side of the body. The vitellaria extend laterally from near the pharynx to within a short distance from the posterior tip; they meet in the post-testicular body space. The uterus extends anteriorly from the ovary to the genital pore, which is situated at the level of the intestinal bifurcation, on the left side of the body. The cirrus pouch overlaps the acetabulum one-quarter to one-half its diameter. The excretory bladder is small and sac-like. The eggs measure 0.07 to 0.09 mm. by 0.05 to 0.06 mm.

Plagioporus serratus sp. nov.

(Fig. 1)

Host: *Hyodon tergisus* (moon-eye)

Members of this species occur in the gall bladder of their host. They are very small, somewhat spindle-shaped flukes, tapering rather abruptly at the posterior tip and narrowing out to a broad neck at the anterior end. The cuticula is characteristically extended to form conspicuous broad-based spines, which cover the entire body. Mature specimens measure from 0.41 to 0.51 mm. in length by 0.14 to 0.18 mm. in width at the widest point, which is usually the region of the acetabulum. The oral sucker is terminal, subspherical and measures from 0.076 to 0.080 mm. in diameter. The acetabulum is situated about the middle of the body. It is subspherical and somewhat larger than the oral sucker, measuring from 0.09 to 0.11 mm. in diameter. There is no obvious pre-pharynx. The pharynx is prominent and is about half as long as the diameter of the oral sucker. The oesophagus is from once to twice the length of the pharynx. The broad intestinal crura extend to within a short distance of the posterior extremity. The testes are obliquely arranged near the posterior tip of the body; the posterior testis is on the mid-line and the anterior testis is immediately to the left of it. They are approximately spherical in outline with smooth edges, and measure from 0.042 to 0.046 mm. in diameter. The ovary is ovate in outline with the long axis in the lateral plane; it is larger than the testes and is situated on the mid-line partly overlapping the anterior testis. The vitellaria extend laterally from the posterior end of the pharynx to the posterior testis. The uterus passes anteriorly to the genital pore situated on the left side of the body slightly

anterior to the level of the intestinal bifurcation. The cirrus pouch overlaps the acetabulum to about half its diameter. The eggs are comparatively large, measuring 0.067 by 0.038 mm. There are usually from four to six eggs in the uterus.

Plagioporus serratus differs from all previously described species of this genus by its extremely small size, and the spinose extensions of its cuticula.

As far as can be determined eight species of *Plagioporus* have been reported from American fishes. Included in this number is *Plagioporus (Lebouria) abducta* (Nicoll, 1909), a parasite described by Linton (1904) from *Bairdiella chrysura*. However, *P. abducta* cannot be retained in the genus *Plagioporus*, as the median position of its genital pore violates the concepts of the genus. A key to the American species of *Plagioporus* is presented below.

1. Cuticula with large spinose extensions; small worms under 0.6 mm. in length.....*P. serratus* sp. nov.
Cuticula smooth, without spinose extensions.....2.
2. Body strongly fusiform; acetabulum broadly ovate with the long axis in the lateral plane, over twice the size of the oral sucker.
P. fusiformis Price, 1934.
Body not strongly fusiform; acetabulum more or less spherical, not over twice the diameter of the oral sucker.....3.
3. Vitellarian follicles numerous in post-testicular body region.....4.
Vitellarian follicles not numerous in post-testicular body region, extending just beyond the testes; testes near posterior tip of body.
P. sinitsini Mueller, 1934.
4. Intestinal crura not extending posterior of the posterior testis.
P. cooperia Hunter and Bangham
Intestinal crura extending posterior of the posterior testis.....5.
5. Vitellarian follicles not extending anterior of the acetabulum.
P. lepomis Dobrovolny, 1939.
Vitellarian follicles extending anterior of the acetabulum.....6.
6. Vitellarian follicles anterior of the acetabulum for the most part confined laterally; acetabulum approximately twice the diameter of the oral sucker.....*P. serotinus* Stafford, 1904.
Vitellarian follicles anterior of the acetabulum meeting medially; acetabulum considerably less than twice the diameter of the oral sucker.....*P. crassigula* Linton, 1911.

Anallocreadium armatum McCallum, 1895

(Fig. 10)

Synonym: *Anallocreadium pearsei* Hunter and Bangham, 1932.

HOSTS: *Ameiurus nebulosus* (bullhead).

Eupomotis gibbosus (sunfish).

This parasite was found rather commonly in the intestine of sunfish, but only five specimens were recovered from the bullhead. All specimens with the exception of one from the sunfish and two from the bullheads contained no eggs.

The mature specimens measure from 1.23 to 2.31 mm. in length by 0.5 to 0.8 mm. wide. They have rounded extremities with the posterior end broader. Spines can be seen on the cuticula of some specimens but only with difficulty. The oral sucker is subspherical. There is a well developed pre-pharynx, a pharynx which measures slightly less than half the diameter of the oral sucker, and a short oesophagus which forks about one-third of the distance between the pharynx and the acetabulum. The intestinal caeca extend well into the posterior portion of the body. The acetabulum is slightly larger than the oral sucker, and is situated in the second quarter of the body. The testes are in tandem arrangement in the third quarter of the body. they are broadly ovate to spherical in outline with smooth margins and may be smaller or larger than the acetabulum. The ovary is small, spherical in outline and situated on the right side of the body immediately anterior of the anterior testis. The vitellaria extend laterally from the posterior margin of the acetabulum to the posterior tip of the body filling in the post-testicular space. The uterus passes anterior from the ovary to the genital pore situated on the mid-line immediately anterior of the acetabulum. Measurements of an average specimen are as follows: length 2.06 mm., width 0.59 mm., oral sucker 0.22 mm. in diameter, acetabulum 0.25 mm. in diameter, ovary 0.13 mm. in diameter, anterior testis 0.24 by 0.31 mm., posterior testis 0.26 by 0.31 mm., egg 0.09 to 0.11 by 0.06 mm.

Hunter and Bangham (4) created the species *Anallocreadium pearsei* for a trematode in the intestine of *Aplodinotus grunniens*. Pearse (11) reported a species of *Anallocreadium* from the same host and from *Eupomotis gibbosus* which he called *A. armatum* but which Hunter and Bangham consider to be *A. pearsei*. The characters used by these authors to separate *A. pearsei* from *A. armatum* are the nature of the margin of the testes, and the comparative sizes of the suckers, of the pharynx, and acetabulum, and of the testes and acetabulum. According to them *A. pearsei* has the oral sucker only slightly smaller than the acetabulum, while *A. armatum* has an acetabulum twice the size of the oral sucker. They consider the testes to be always larger than the acetabulum in *A. pearsei* and usually smaller in *A. armatum*. They state the length of the pharynx to be about one-third the diameter of the acetabulum in *A. armatum* and one-half the diameter in *A. pearsei*. Finally, they consider the testes to have smooth margins in *A. armatum* and lobed margins in *A. pearsei*.

The specimens recovered in the present survey show characters of both species. The oral sucker is only slightly smaller than the acetabulum which is characteristic of *A. pearsei*, but the testes are smooth-margined, a characteristic of *A. armatum*. The length of the pharynx varies from one-third to one-half the diameter of the acetabulum, and the testes range in size from

smaller, to considerably larger than, the acetabulum. It appears, therefore, that there is but one valid species of *Anallocreadium*, and that *A. pearsei* must be considered a synonym of *A. armatum*.

Bunodera eucaliae (Miller, 1936)

Synonym: *Bunoderina eucaliae* Miller, 1936

Host: *Eucalia inconstans* (stickleback).

This parasite, originally referred to a new genus by the author because of the character of its uterus which is tubular rather than sacculate, resembles *Bunodera sacculata* Van Cleave and Mueller (15) in so many characters that it seems more consistent to place it in the same genus. The tubular uterus in *B. eucaliae* probably points out the relationship of *Bunodera* and *Crepidostomum* and may indicate the direction of the evolutionary trend.

Three species of *Bunodera* have been reported from North America. They can be distinguished as follows:

1. Vitellaria extending laterally from anterior of the acetabulum to near the posterior tip of the body.....*B. luciopercae* Müller, 1776.
Vitellaria extending laterally from anterior of the acetabulum to about the middle of the body length.....2.
2. Vitellaria not extending to the level of the testes; acetabulum larger than oral sucker; mature forms with tubular uterus..*B. eucaliae* Miller, 1936.
Vitellaria extending to the level of the testes; acetabulum not larger than oral sucker; mature forms with sacculate uterus
B. sacculata Van Cleave and Mueller, 1934.

FAMILY PLAGIORCHIDAE

Parastiotrema ottawanensis gen. et. sp. nov.

(Figs. 4 and 5)

Host: *Ictalurus punctatus* (channel catfish)

Only three specimens of this small trematode were recovered from the intestine of the channel catfish. One of the specimens was contracted to such an extent that it could not be studied.

Members of this species are small muscular worms, pointed at the posterior tip and more or less rounded anteriorly. The middle part of the body is parallel-sided. The cuticula is covered with very fine spines which are more prominent anteriorly and gradually disappear toward the posterior part of the body. One of the specimens is longer and narrower than the other, measuring 0.72 by 0.22 mm.; the other measures 0.58 by 0.27 mm. The oral sucker is spherical to subspherical in outline; in one specimen it measures 0.092 and in the other 0.108 mm. in diameter. The acetabulum is approximately the same size as the oral sucker. It is situated in the first or second quarter of the body, depending on the state of contraction of the body. The pharynx is about half as long as the oral sucker. There is no apparent pre-

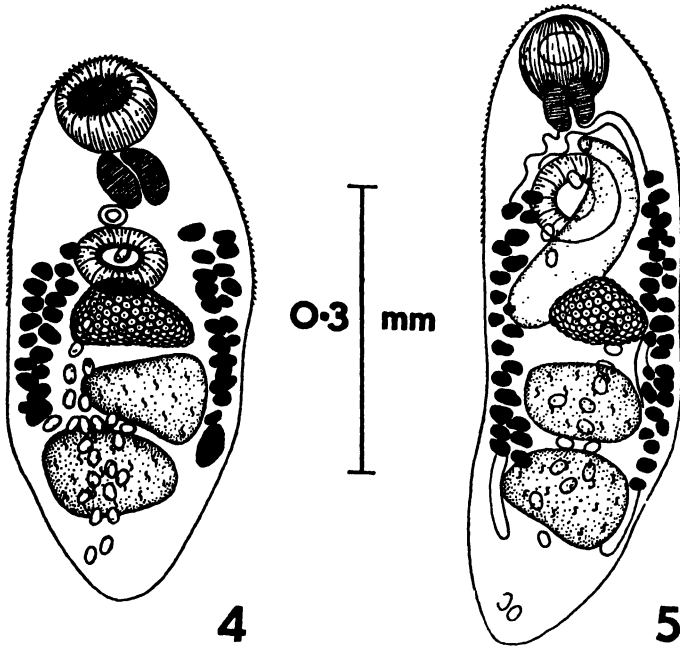


FIG. 4. *Parastiotrema ottawanensis*, ventral view. FIG. 5. *P. ottawanensis*, dorsal view.

pharynx nor does there appear to be an oesophagus. The intestinal crura lead to the posterior border of the posterior testis. The testes are comparatively large, with smooth or slightly irregular margins; they are roughly triangular in shape with the long axis in the lateral plane. They measure up to 0.08 mm. in width, and are in tandem arrangement with the anterior displaced slightly to the left. The ovary is situated between the anterior testis and the acetabulum. It is triangular in shape and somewhat smaller than the testes. The uterus passes back to the posterior tip of the body. The vitellaria extend laterally from the anterior margin of the acetabulum to the posterior margin of the posterior testis. The genital pore is situated on the mid-line immediately anterior of the acetabulum. The cirrus pouch is long and broad extending posteriorly to overlap the ovary. The eggs measure 0.025 by 0.017 mm.

The genus *Parastiotrema* appears to be closely related to *Astiotrema* Looss, 1900, and *Alloglossidium* Mueller, 1930. Like *Astiotrema* it has a very large cirrus pouch which differs, however, in that, unlike the cirrus sac in that species, it does not have the terminal end particularly thin. The large triangular shaped genital organs are quite distinct from those exhibited by the species of *Astiotrema* as is the character of the alimentary tract. It differs from *Alloglossidium* mainly in the character of the alimentary tract, and in the shape and size of the testes and ovary.

Generic Diagnosis

Plagiorchiinae; small parallel-sided forms, more or less pointed at the posterior extremity, and rounded at the anterior end. Cuticula with minute spines; alimentary tract with well developed oral sucker and pharynx, but with no apparent pre-pharynx or oesophagus. Testes and ovary large, roughly triangular in outline; testes in the posterior half of the body in tandem arrangement; ovary anterior of the anterior testis; uterus extending back to the posterior tip of the body. Cirrus sac very large and broad, extending back to the posterior margin of the ovary; vitellaria laterally arranged from the acetabulum to the testis; occurring in the intestine of fishes.

Alloglossidium geminus Mueller, 1930

(Fig. 9)

Host: *Ameiurus nebulosus* (bullhead).

Specimens of this species are common in the intestine of the bullhead in this vicinity. They range from 0.68 to 1.27 mm. in length.

Alloglossidium corti Lamont, 1921

(Figs. 7 and 8)

Host: *Ictalurus punctatus* (channel catfish).

This species was very commonly recovered from the intestine of the channel catfish. Mature specimens ranged from 0.53 to over 2.0 mm. in length. The smaller specimens have the genital organs and the acetabulum situated proportionately further back in the body, and the body is comparatively wider. The presence of intermediate forms, however, shows the two types to be of the same species. As pointed out by van Cleave and Mueller (15) this species can be separated from *A. geminus* by the character of the vitellaria which extend anterior of the acetabulum in *A. corti*, and not anterior of the anterior margin of the acetabulum in *A. geminus*.

FAMILY HETEROPHYIDAE

Cryptogonimus chyli Osborn, 1903

(Fig. 6)

Hosts: *Ambloplites rupestris* (rock bass)

Micropterus dolomieu (small-mouth black bass)

Only a few specimens of this species were obtained from the above mentioned hosts.

FAMILY AZYGIIDAE

Azygia angusticauda Stafford, 1904

Hosts: *Stizostedion vitreum* (doré)

Micropterus dolomieu (small mouth black bass)

Three specimens of this species were recovered, one from the intestine of the bass, and the other two from the intestine of the doré. The specimen

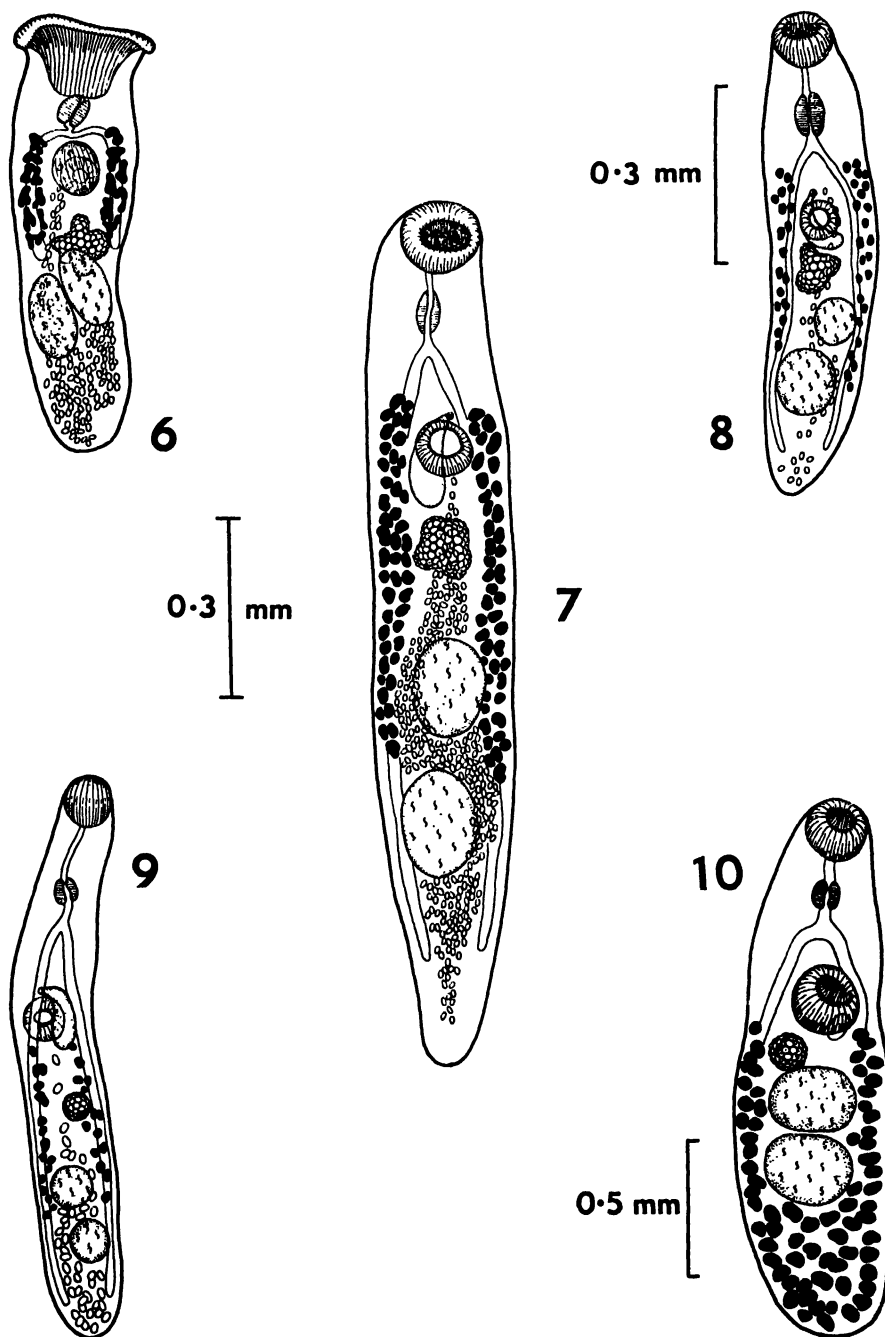


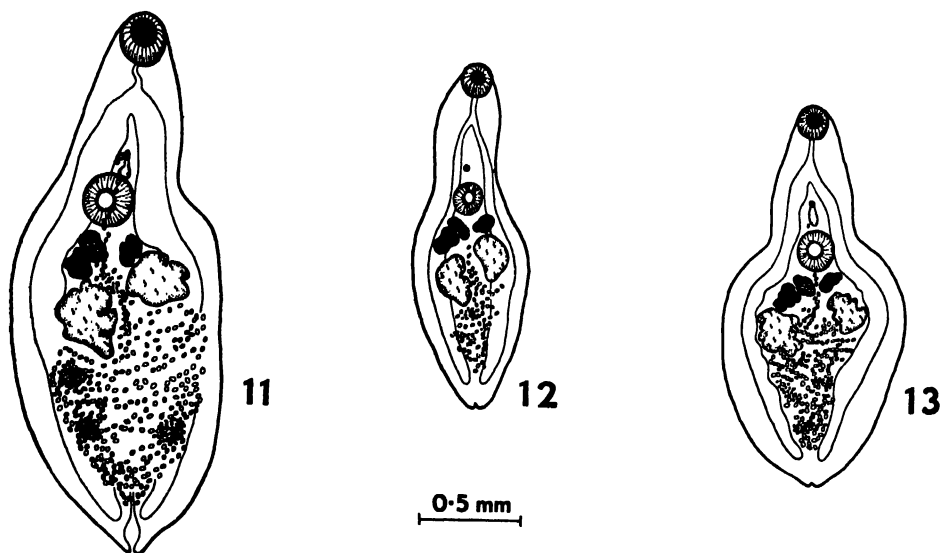
FIG. 6. *Cryptogonimus chyli*, dorsal view. FIGS. 7 AND 8. *Alloglossidium corti*, ventral views. FIG. 9. *A. geminus*, dorsal view. FIG. 10. *Anallocreadium armatum*, dorsal view.

from the bass measured slightly less than 2.0 mm. in length, while those from the doré measured 11 and 12 mm. respectively. Van Cleave and Mueller (15) reported similar large specimens from the doré and it is possible that they may represent a distinct subspecies.

FAMILY GORGODERIDAE

Genus *Phyllodistomum* Braun, 1899

As far as can be determined, up to the present time seven species of *Phyllodistomum* have been reported from American fishes. During the present survey, a species of *Phyllodistomum* was recovered from the urinary tubules of *Catostomus commersonii* and upon careful examination it proved to be heretofore undescribed. The name *P. lysteri* is proposed for this species in honour of the late L. L. Lyster by whom the first specimens of this species were collected.



FIGS. 11, 12, 13. *Phyllodistomum lysteri*, ventral view.

Phyllodistomum lysteri sp. nov.

(Figs. 11, 12, and 13)

Host: *Catostomus commersonii* (common sucker)

The body of this species is flattened and made up of a narrow anterior region and a broad posterior part; the approximate ratio of the anterior to the posterior part of the body is as 1 : 2. The posterior end of the body is obtusely pointed and may show a small notch. The cuticula is smooth, and the body margins do not show any crinkling. Specimens range from

1.75 to 3.2 mm. in length by 0.5 to 1.0 mm. in width. The oral sucker is situated at the junction of the narrow and wider portions of the body; it may be the same size or very slightly larger than the oral sucker, measuring from 0.20 to 0.26 mm. in diameter. There is no pharynx. The oesophagus is approximately the same length as the oral sucker; it divides into two broad intestinal crura which extend to within a short distance of the posterior tip of the body. There are two large testes with irregularly lobed margins, situated in the middle third of the body. They are obliquely arranged with the left testis usually more anterior than the right, although this arrangement may be reversed. The lobed ovary is about one-third the size of the testes and is situated immediately anterior of the more posterior testis. The uterus fills the posterior part of the body between the intestinal crura, finally leading to the genital pore which is situated on the mid-line, a short distance above the acetabulum. There are two solid, lobed, vitellarian bodies, lying between the acetabulum and the genitalia, in tandem arrangement. The uterus contains what appear to be two types of eggs. One is a typical egg with an oval outline and a well developed egg shell, measuring 0.031 to 0.036 mm. by 0.018 to 0.021 mm. The other is spherical in outline and does not show a well developed egg shell. It is larger than the more typical egg measuring 0.032 to 0.052 mm. in diameter, and stains readily with alum carmine. The uterus of every mature specimen shows these two types of eggs.

Phyllodistomum lysteri is readily distinguishable from *P. pearsei*, *P. carolini*, *P. staffordi*, *P. lacustri*, and *P. fausti* by the fact that the oral and ventral suckers are not obviously different in size in this species, while they do show obvious difference in size in the last five species. It is further differentiated from *P. fausti* by not having the post-acetabular body widest in the middle and by the space between the testis, from *P. staffordi* and *P. carolini* by not having the post-acetabular part of the body discoidal, and from *P. lacustri* by having a smooth cuticula. It most closely resembles *P. superbum* and *P. folium*. However, it is distinguished from these two species by the more anterior position of its gonads and by the ratio of the pre- to the post-acetabular regions of the body which in *P. lysteri* is about 1 : 2, whereas in the *P. superbum* and *P. folium* it is 1 : 1.25. Furthermore it can be differentiated from *P. folium* by its larger size. and from *P. superbum* by the fact that the body margins are not crinkled.

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COLOUR OF MEAT

III. AN IMPROVED COLOUR COMPARATOR FOR SOLIDS¹

BY C. A. WINKLER,² W. H. COOK,³ AND E. A. ROOKE⁴

Abstract

A photoelectric colour comparator, previously described (Can. J. Research, D, 17: 1-7. 1939) has been improved to permit greater precision and more rapid operation. Measurements on meat indicate that the degree of precision attainable with the new comparator is determined primarily by sampling, rather than instrumental, errors.

Introduction

An objective colour comparator suitable for estimating the colour of solids was described in the first paper of this series (1). The results obtained with this instrument in subsequent investigations on the colour (2, 4) and colour stability of bacon (3, 5) led to the construction of an improved model. Enquiries requesting a more detailed description and other information regarding the earlier apparatus prompted the preparation of this article describing the new instrument.

The new apparatus, like the old, is based on the measurement of the amount of each main component of white light, namely blue, green, and red, scattered by the test sample and expressed as a percentage of that scattered by a standard white surface. Such measurements naturally yield less information than those made with a spectrophotometer, but have the advantage that they can be made more rapidly. This feature enables measurements to be made on labile materials without appreciable change, and facilitates making a sufficient number of measurements to estimate the variability present in such biological materials as meat. The equipment was originally designed to place the subjective visual estimates of colour and colour stability on an objective basis. Subsequent papers (3, 4, 5) have shown that not only was the older instrument capable of detecting significant differences in the colour of different samples, but it was also possible, by statistical studies of a large number of measurements, to relate the scatter of the individual colour components to the composition of the bacon with respect to certain constituents.

Description of Apparatus

The new instrument is fundamentally the same as the earlier model. The most important changes are: baffle plates to minimize internal reflections; diaphragm for controlling light intensity, thus enabling the light source to be

¹ Manuscript received Aug. 12, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories Ottawa. Issued as Paper No. 53 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 960.

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operated at fixed voltage and avoiding major change in colour quality; a more sensitive photo-cell, and a more sensitive galvanometer. Mechanically the apparatus was much improved, permitting greater accuracy and more rapid operation.

A photograph of the apparatus is shown in Fig. 1 and a diagrammatic sketch in Fig. 2. It is impossible to give complete details of construction in this article but the information contained in the figures and the following brief description are considered adequate to permit the construction of a comparable instrument wherever the necessary facilities are available.

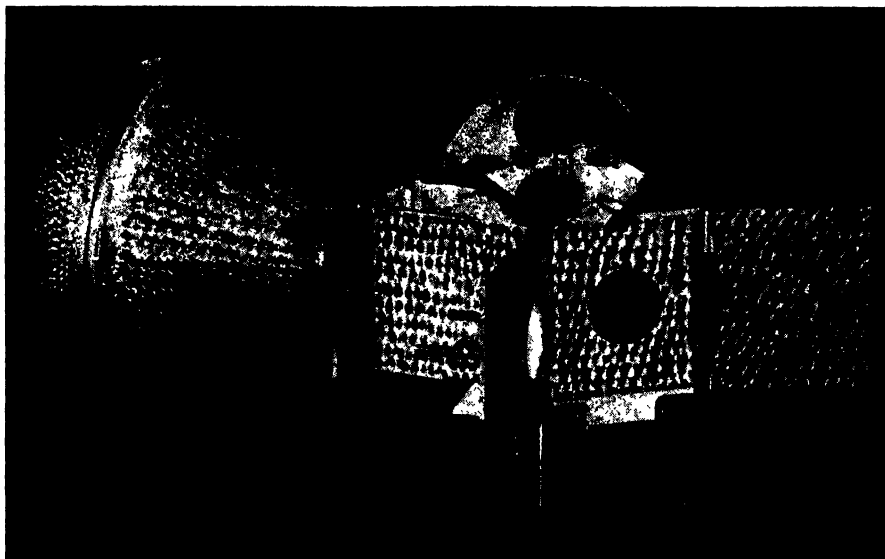


FIG. 1. *Colour comparator showing general arrangement, lamp housing, reference and test sample holders, and selective filter holder.*

The two sections of the lamp housing, *A*, and the adapter section, *K*, are dural castings with drilled and tapped flanges for attachment. The remainder of the apparatus is constructed from flat or angle aluminium fastened with screws to facilitate assembly.

The two sections of the lamp housing are water jacketed, and a continuous circulation of cooling water is maintained during operation. The hemispherical portion at the back is polished on the inside. A semi-circular opening is provided between the two halves of the lamp housing to accommodate a No. 2 photo-flood bulb. The walls of this portion of the housing are thicker and shaped to permit the attachment of an aluminium shield, supporting the lamp socket, to the housing with screws. As the photo-flood bulb must be screwed into the socket from the inside, the two sections of the housing must be separated when the bulb is changed, but as this is seldom necessary it is not a troublesome operation.

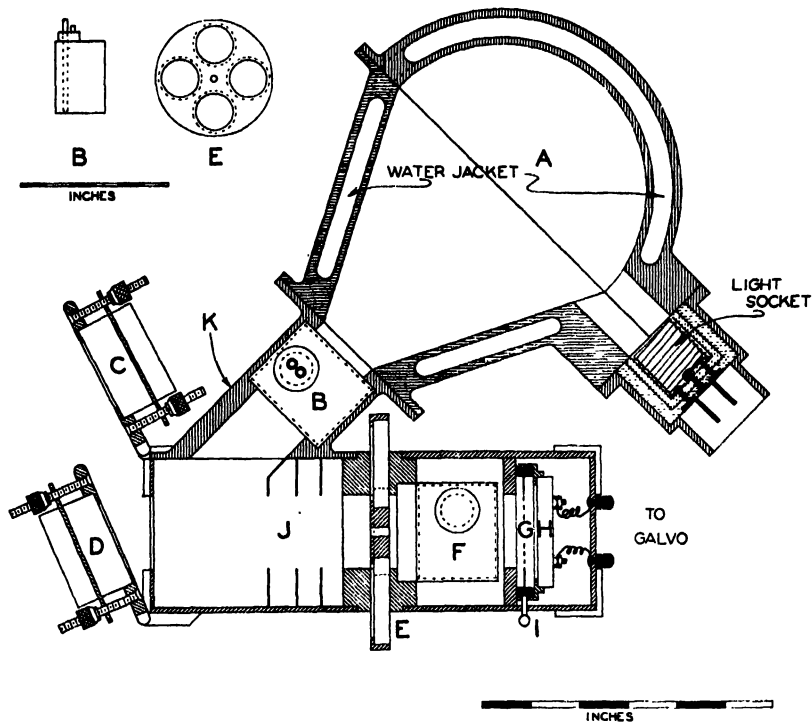


FIG. 2. Diagrammatic sketch of colour comparator (plan view).

The adapter section, *K*, is of rectangular cross section and flanged for attachment to the lamp housing. The end furthest removed from the housing is shaped to permit attachment to the metering portion at an angle of 45° . An oval opening, 1.5 in. high and 1.06 in. wide, is provided through the centre of this block, to allow the incident light at an angle of 45° to fall uniformly on the $1\frac{1}{2}$ in. diameter circular opening containing the test sample. A rectangular recess, open at the top, is provided at right angles to the path of the light to accommodate the water cell, *B*, for removing part of the infra-red radiation from the incident light beam. This cell may be glass but a plastic (plexiglass) has been found more convenient. The cap for this cell is provided with two tubes for the circulation of cooling water (tap) during the course of the measurements.

The reference standard and test sample holders, *C* and *D*, consist of removable doors hinged on opposite sides of the rectangular part of the instrument. Each door consists of two plates arranged to hold the sample between them by clamping. The outer plate is solid, lightproof, and moves back or forth on the clamping screws. The inner plate is fixed and has a centrally drilled hole $1\frac{1}{2}$ in. in diameter to expose the material. A similarly drilled plate member, darkened on the inside, forms the back of the instrument. The test sample is usually placed on an aluminium plate, or in a short cylinder with a locating pin if the exact test position is important, and covered with

a clean glass slide before clamping in the holder. A similarly prepared cell containing magnesium carbonate is used as a reference standard.

Light scattered by the sample passes the baffles, *J*, and falls on a glass filter held in a rotating stainless steel holder, *E*, fitted in a light-tight manner into a slot in an aluminium block. Stainless steel was found to produce less friction and stand up better to the wear imposed on this member than an aluminium holder. Following the selective filters is a cell containing a 1% copper sulphate solution. This cell is identical with the water cell except that it has an ordinary screw cap since openings for water circulation are unnecessary. The iris diaphragm, *G*, is placed immediately in front of the photo-cell, *H*, and is adjustable by a short lever, *I*, at the side of the instrument. The centre inner surface between the sample holders and the photo-cell must be light-tight and finished with a flat black paint.

Accessory Equipment

The following accessory parts, obtainable from the stated sources, have been found suitable after considerable experimentation.

Plexiglass cells—made to dimensions and specifications by Stricker-Brunhuber Corp., New York, New York.

Selective filters—

Blue (400–500) Jena BG12, or Corning Signal Blue No. 556.

Green (500–600) Wratten W58, or a combination of Corning Light Theatre Blue No. 502 and H. R. Noviol No. 352.

Red (600–750) Jena OG3 or Corning H. R. Lantern Red No. 244.

Iris diaphragm—Leitz catalogue No. 8503.

Photo-cell—Visitron F-2A. G.-M. Laboratories Inc., Chicago.

Galvanometer—Box type Catalogue No. 4625, Rubicon Co., Philadelphia.

Transformers—The Variac transformer, General Radio Co., Cambridge, Mass., used for controlling the light source is generally unnecessary if an iris diaphragm is used, but may be required in special circumstances. Where line voltage is subject to some variation a constant voltage transformer will improve the accuracy and facilitate rapid operation. Suitable types are available from the Sola Electric Co., Chicago.

Operation

The manipulation of the new machine is simple and rapid. The filter holder is rotated until the desired filter is in position, the standard white holder swung into place, and the galvanometer deflection adjusted to the desired value with the diaphragm. The standard white holder is then swung out, the test sample swung into position and the galvanometer deflection observed. This sequence of operations can be performed in less than a minute for each colour filter used. In fact, with reasonably constant voltage, the three colour components can be measured in two minutes, including the

time required to place the test piece in the holder and remove it. This is a much more rapid rate than attainable with the older instrument and greatly facilitates the measurement of colour in routine operations such as process control. The apparatus is not only suitable for measuring the colour of such solids as meat, but has also been applied to ground materials, such as flour.

Accuracy

The accuracy of the new model can be compared with that of the old model, or with the error of sampling such biological materials as meat, from the results presented in Table I. The mean values and the standard errors of a single measurement were computed from duplicate measurements on some 30 to 40 samples picked at random from routine observations. The measurements on bacon with the old and new models were not made on the same samples and this may account for a portion of the difference between the mean values obtained with the two instruments. The greater part of this difference, however, is attributable to the leakage of light in the older model. The "dark" constant obtained with a standard black, as a test sample, was about 22% of that obtained from the standard white in the earlier model, and only 4% in the new model. This reduction in the dark constant was brought about largely by the insertion of the baffle plates, *J*, in the new instrument. The difference of 18% accounts for most of the difference between the corresponding means reported in Table I.

TABLE I
PRECISION OF NEW COLOUR COMPARATOR AS COMPARED WITH THAT OF OLDER INSTRUMENT
AND SAMPLING ERROR

Colour component	Old instrument		New instrument				
	Bacon only		Bacon		Pork		
	Mean	Std. error (instrument)	Mean	Std. error (instrument)	Mean	Std. error (sampling)	Std. error (instrument)
Initial colour							
Red	43.2	1.41	25.4	1.00	25.7	1.48	1.13
Green	31.1	0.87	14.5	0.45	16.2	1.12	0.77
Blue	28.3	0.61	11.5	0.36	11.9	0.74	0.64
Brightness	-	-	-	-	53.9	3.37	2.29
Colour stability*							
Red	5.35	1.30	4.03	0.60	2.19	0.43	1.04
Green	3.66	1.03	2.70	0.50	0.77	0.53	0.92
Blue	2.57	0.89	2.50	0.40	0.87	0.37	0.63
Brightness	-	-	-	-	3.83	1.33	2.22

*Mean change of scatter, usually a decrease from the initial value.

The standard error of the instrument was computed by statistical methods from the difference between duplicate observations. The two values were obtained by cutting a piece of meat and making a single measurement on each of the two surfaces so produced. This practice tends to exaggerate the instrumental error by including the error of subsampling the test surface, and duplicate measurements on the same surface can be checked within narrower limits. The results of measurements of both the initial colour and colour stability of bacon show that the standard error of the new instrument is about half of that observed on the old instrument.

When the standard error of the initial colour measurement is expressed as a percentage of the mean for the corresponding colour component, the accuracy varies from about ± 2 to 4%. This may appear to represent satisfactory precision, but it must be remembered that a difference of 10 to 20% in scatter represents the difference between a satisfactory and wholly unacceptable colour as judged by visual standards. In these circumstances it might appear that a still higher instrumental precision would be desirable for making fine distinctions, without the need for tedious replication.

In connection with an investigation into the storage of pork, provision was made for estimating both the sampling and instrumental errors by making duplicate measurements on duplicate pieces of pork taken from the same part of the same carcass and treated identically throughout. An analysis of variance showed that the combined sampling and instrumental errors were significantly greater than the instrumental error alone. It appears therefore that the sampling error is the source of variation limiting the accuracy of initial colour measurements and that little, if any, increase in precision would be accomplished by further refinements in the instrument.

These results were analyzed further with the object of determining the method of measurement capable of yielding the greatest precision for a limited number of observations. The last two columns show the standard error of sampling and instrumental measurements independently, on a single observation basis. The sampling error for the initial colour estimations is in all instances larger than the instrumental error. It is therefore evident that four single instrumental observations on four separate samples taken from the test material would yield a more accurate estimate of the true colour than duplicate instrumental readings on two samples. In other words, the instrumental error is not the factor limiting the accuracy of colour measurements on meat.

A somewhat different situation exists with respect to the error of estimating the change in colour during colour stability measurements (lower section, Table I). Although the standard instrumental error is of the same order as that for the initial colour measurement, the change in colour scatter is much smaller than the original scatter and is consequently estimated with much less precision on a percentage basis. As can be seen from Table I, the sampling error of colour stability measurements is less than the instru-

mental error. In these circumstances precise estimates of colour stability can be obtained with the present equipment only by adequate replication.

From the standpoint of practical colour measurements on a particular material, meat, flour, etc., the most important factor is to select colour filters that will yield the maximum information on colour quality for the material in question. In this respect the new instrument is the same as the old, since the filters separating the three broad colour bands are the same. Spectroscopic studies of meat are now under way with the object of determining the practicability of using more selective filters. Should such a modification be desirable, it is possible that the use of extremely narrow colour bands might require more sensitive measuring equipment.

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STUDIES ON *OESTRUS OVIS* L.¹

BY A. MURRAY FALLIS²

Abstract

The incidence of infection of *Oestrus ovis* larvae in lambs has been determined. Flies were obtained from larvae which pupated in the laboratory. The three larval stages of the fly were studied and characteristic features illustrated. Information relative to the rate of growth of the larvae was obtained from a routine examination of infected animals and experimental infection of lambs. The effect of the parasites on these experimentally infected animals was noted.

Introduction

The investigation was concerned with a study of the habits and development of the parasitic stages of *Oestrus ovis*. The incidence of infection was determined from an examination of sheep and lambs, one year old and under, received at a local abattoir from scattered points in Ontario and Western Canada. The rate of development was studied by the routine examination of slaughtered animals throughout the year and by experimental infection of parasite-free lambs.

Incidence of Infection

Of 698 animals examined, 50% were infected with larval stages of the parasite. A higher incidence would be obtained if the total included only the older animals, for over 90% of the lambs examined from August to May were infected.

Length of Life of Flies

Flies (Fig. 1) were reared from larvae which pupated in sand in the laboratory. Twelve flies were obtained in this way between May 2 and September 24, which are the first and last dates of emergence. Eight of the flies kept at room temperature lived an average of 16 days, one living for 28 days and two for 10 days only. This may be significant, for if flies remain alive and active for two weeks in their natural environment, a relatively small population might be capable of producing a high incidence of infection.

Mitchell and Cobbett (2) demonstrated that small, first-stage larvae could be expelled from female flies by pressing their abdomens. Hadwen has informed the writer that he used the same method to obtain larvae of a related fly, *Cephenomyia nasalis*. It seems likely, therefore, that *Oestrus ovis* is viviparous although Seguy (5) claims it is oviparous as well.

Larval Stages

The smallest larvae found measured 0.8 to 1.0 mm. in length following fixation in hot alcohol, whereas the largest measured 24 mm. The former were taken on September 2 and November 25 respectively, the latter on

¹ Manuscript received August 14, 1940.

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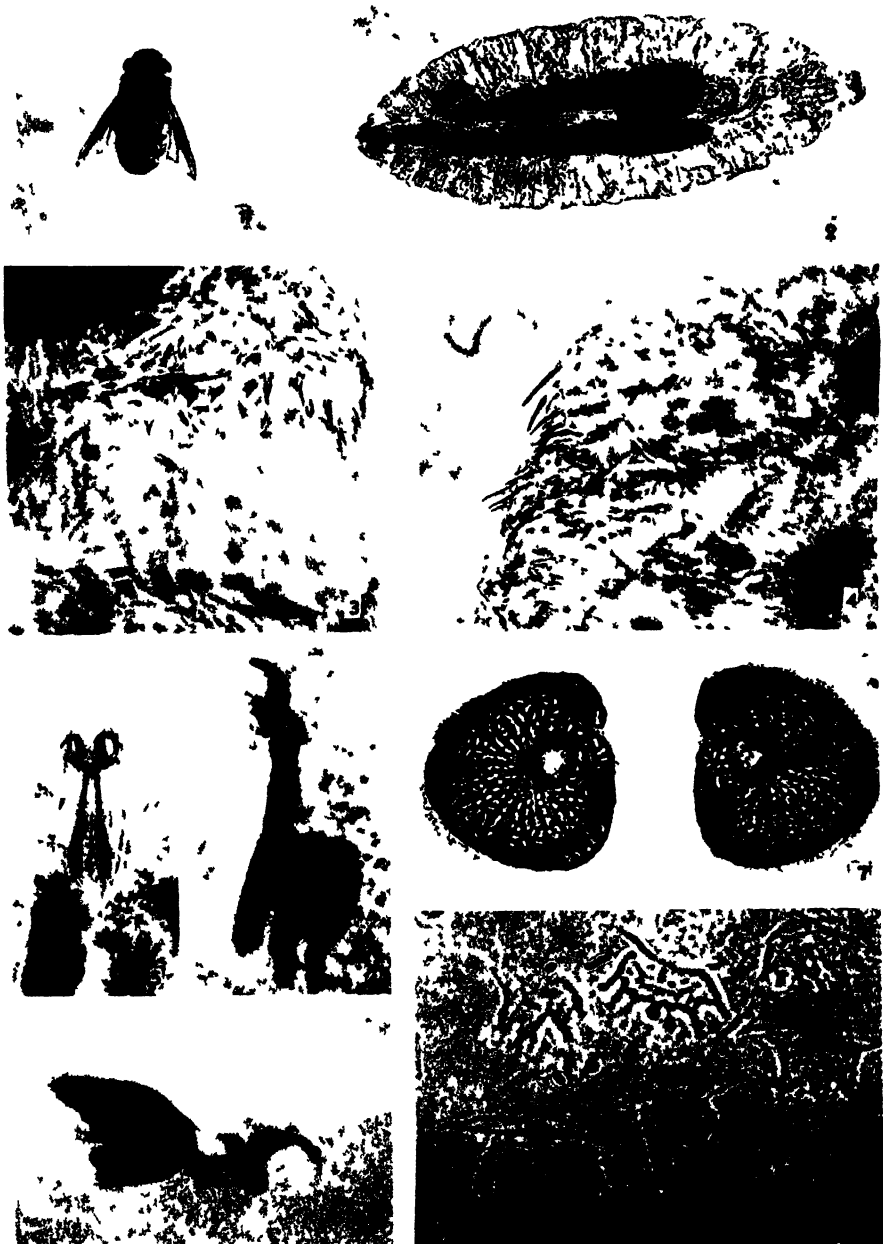


FIG 1 Adult fly *Oestrus ovis* $\times 1\frac{1}{2}$
 FIG 2 Ventral view of first instar larva. Note row of curved spines at posterior $\times 40$
 FIG 3 Typical spines on ventral surface of segments of first stage larva $\times 300$
 FIG 4 Typical spines at ventro lateral margins of segments of first stage larva $\times 300$
 FIG 5 Ventral view of pharyngeal sclerites and oral hooks of first stage larva $\times 80$
 FIG 6 Lateral view of pharyngeal sclerites and oral hooks of first stage larva $\times 400$
 FIG 7 Posterior spiracles of second stage larva $\times 80$
 FIG 8 Lateral view of pharyngeal sclerites and oral hooks of second stage larva $\times 40$
 FIG 9 Typical spinous plates on the ventral surface of the middle segments of second stage larva $\times 400$

All the photographs were made from unetched negatives

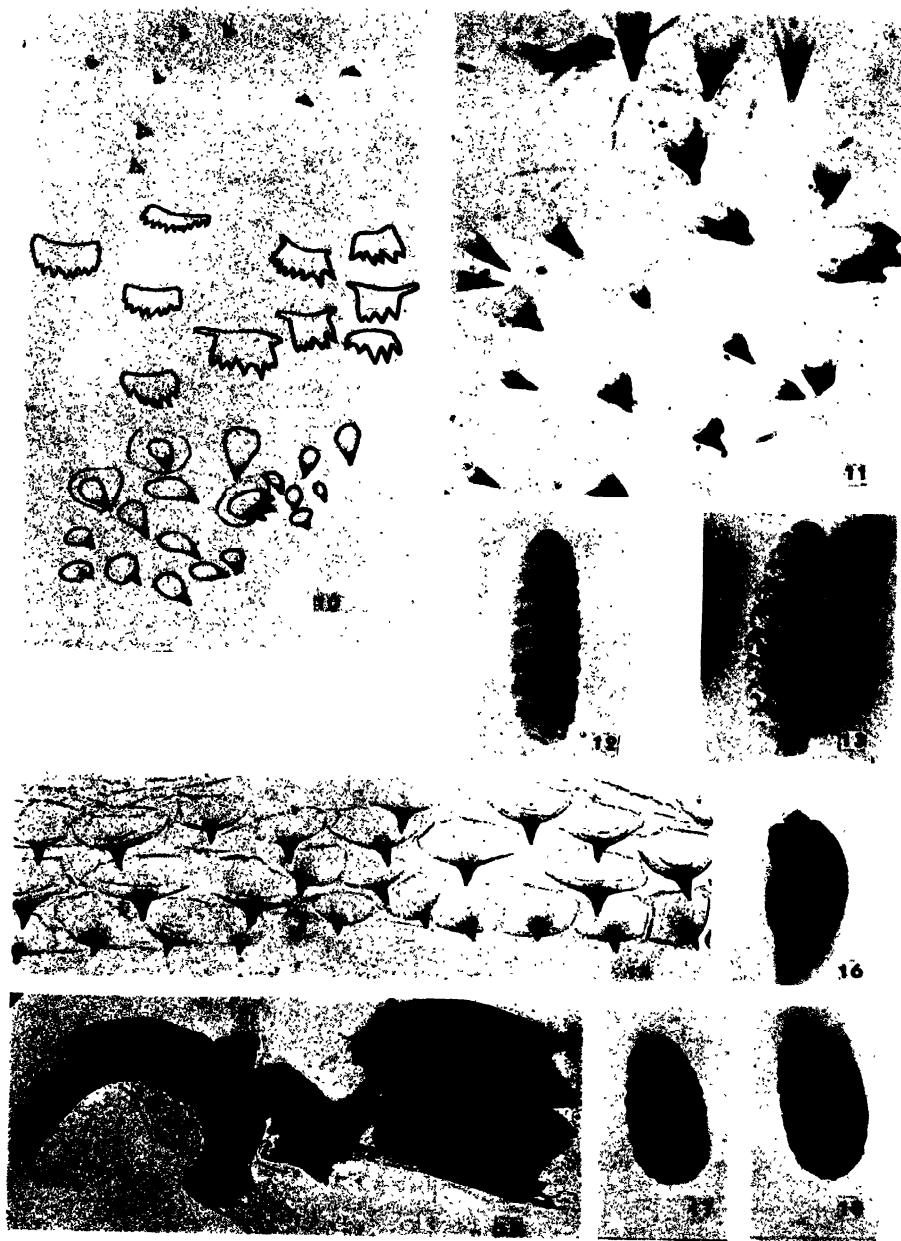


FIG. 10. Outline drawings of typical spines on ventral surface of second-stage larva. Those on the anterior segment are shown in the upper part of the figure, those on Segments 2 to 10 in the middle part of the diagram, and those on Segment 11 in the lower part of the figure. $\times 400$.

FIG. 11. Photomicrograph of typical spines on ventral surface of 11th segment of second-stage larva. $\times 400$.

FIG. 12. Dorsal view of third-stage larva. $\times 1\frac{1}{2}$.

FIG. 13. Ventral view of third-stage larva. $\times 1\frac{1}{2}$.

FIG. 14. Typical spines on ventral surface of third-stage larva. There are eleven rows of these. $\times 40$.

FIG. 15. Lateral view of pharyngeal sclerites and oral hooks of third-stage larva. $\times 40$.

FIG. 16. Lateral view of pupa. $\times 1\frac{1}{2}$.

FIG. 17. Ventral view of pupa. $\times 1\frac{1}{2}$.

FIG. 18. Dorsal view of pupa. $\times 1\frac{1}{2}$.

March 16. First-stage larvae (Fig. 2) attained a maximum length of 5.0 mm. Characteristic features of this instar are the transverse rows of spines on the ventral surface of the segments (Fig. 3), the longer spines on the latero-ventral margins (Fig. 4), the row of curved spines on the posterior segments (Fig. 2), and the size and structure of the pharyngeal sclerites (Figs. 5 and 6).

Second-stage larvae measured 3.0 to 14.5 mm. The posterior spiracles of this stage are very apparent (Fig. 7). The pharyngeal sclerites and oral hooks are larger and have undergone some change in form (Fig. 8) from those of the previous stage. According to Portchinsky (3), quoted by Rodhain and Bequaert (4), the first-stage larvae, after penetrating to the sinuses, lose their spines and change to the second-stage. This he considers is a state of rest in which they pass the winter. It is evident from the present studies, however, that winter may be passed in the first-stage as well. Moreover, second-stage larvae are not devoid of spines (Figs. 9, 10, and 11), although they differ from those on first instar larvae. The most anterior and posterior rows of spines have a single spinous process (Figs. 10 and 11), whereas those on the intervening segments have the form of small plates terminating in a serrated edge (Figs. 9 and 10).

Third-stage larvae measured 9.5 to 24.0 mm. in length (Figs. 12 and 13). The eleven rows of spines on the ventral surface (Fig. 14) are prominent and all have a similar shape. The posterior spiracles are larger and more heavily chitinized than those of the second instar larvae. The pharyngeal sclerites are also larger and show further modification (Fig. 15).

Many of the lambs examined were harbouring all three stages of the parasite. This confirms an observation made almost 150 years ago by Clark (1) who wrote ". . . quite young and full-grown larvae may be found in the sinuses at the same time."

Rate of Development of Larvae

The rate of development of the larvae varies within wide limits. Spring lambs in Ontario are probably seldom exposed to the parasite before the beginning of May. No flies were taken in the field at this time but one was reared in the laboratory on May 2. On July 29 a larva, which formed a pupa from which a fly emerged, was recovered from the sinus of a lamb autopsied on this date. Thus it would seem the parasite cycle in spring lambs may require about three months in early summer. This confirms the work of Mitchell and Cobbett (2) who found the complete development of the parasitic stage in spring lambs in Texas and New Mexico required $2\frac{1}{2}$ to $3\frac{1}{2}$ months.

A study of natural as well as artificial infections indicates that development may not always be so rapid. This was shown by the presence of larvae less than 2 mm. long in the lambs examined throughout the fall and winter months when the temperature was such as to preclude the possibility of recent infection. On September 16, for example, 97% of 46 larvae obtained from five lambs examined were less than 5 mm. in length. Excluding one larva

which was 12 mm. long, the average length for the remaining 45 was 1.9 mm. On December 16, if we exclude one larva of 7.5 mm. from 75 obtained from five animals, the average for the remainder, which were all less than 3 mm., was 1.8 mm. On March 16, 43 larvae were recovered from two lambs. Three of these measured 15.3, 7.8, and 5.6 mm., respectively. The remainder were all less than 4 mm. in length, the average being 2.2 mm. A summary of the infection found in lambs at different times of the year is given in Table I.

TABLE I

—	Total number of larvae	Percentage of larvae in different size groups			
		0-5 mm.	6-10 mm.	11-15 mm.	Over 15 mm.
March	74	10.8	46.0	10.8	32.4
April	81	60.5	14.6	12.2	12.2
May	62	43.5	24.2	22.6	9.7
June		No parasites found in 50 spring lambs examined			
July	93	75.4	7.5	14.0	3.2
August	275	77.1	4.0	9.1	9.8
September	169	100.0—	+	0	0
October	89	97.5	2.5	0	0
November	380	98.6	1.4	0	0
December	209	98.5	1.5	0	0
January	62	96.7	3.3	0	0
February	62	96.7	3.3	0	0
March	93	94.6	3.2	1.7	1.7
April	64	78.0	7.8	11.7	3.0
May	50	54.0	16.0	20.0	10.0

The large number of first-stage larvae and the small number of third-stage larvae found from September to February is apparent. It is also seen that the number of second- and third-stage larvae recovered is much smaller than the number of first-stage larvae. The majority of the small larvae are found in the nares and many of them are probably sneezed out, whereas the larger larvae escape as they are usually found in the sinuses. Many first-stage larvae would be overlooked if the sinuses only were examined.

It was thought that the environmental temperature of the host might affect the rate of growth of the larvae. Three infected lambs were moved from an outdoor, midwinter temperature to a heated building, other infected lambs were left outdoors. One of the three lambs was left as a control. The second was given additional infection by transferring larvae less than 2 mm. long into its nostrils the same day as they were removed from infected animals. The third was given additional infection weekly by transferring small larvae to it. The first two lambs were slaughtered five weeks after being brought indoors and the third five weeks later. Control animals from outside were examined at the same time. The results are given in Table II.

An additional experiment was carried out in which two spring lambs, one month old, were infected by transferring larvae less than 2 mm. long to their nostrils. The lambs were kept in a heated building. Lamb No. 1 died

TABLE II

—	Time between infection and slaughter, in weeks	Number of larvae recovered	Maximum size, mm.	Minimum size, mm.	Average size, mm.
Lamb No. 1 (control)	5	5	2.5	2.2	2.3
Lamb No. 2	5	8	2.5	2.2	2.3
Outdoor control to No. 2	?	26	7.5	1.9	2.9
Lamb No. 3	10	50	18.0	1.5	5.0
Outdoor control to No. 3	?	15	5.6	1.9	2.5
Outdoor control to No. 3	?	28	15.3	1.8	2.4

of pneumonia eight weeks after being infected. It was autopsied, but not immediately following death so that some larvae may have escaped from it. Lamb No. 2 was autopsied five weeks after being infected. The results are indicated in Table III.

TABLE III

—	Time between infection and slaughter, weeks	Number of larvae recovered	Maximum size, mm.	Minimum size, mm.	Average size, mm.
Lamb No. 1	8	10	7.7	2.1	4.9
Outdoor control to lamb No. 1	?	17	21.0	1.9	11.1
Lamb No. 2	5	14	17.0	1.8	6.8
Outdoor control to lamb No. 2	?	27	17.0	2.0	7.0

It appears from the above-described experiments, therefore, that an increase in the temperature of the host's environment does not necessarily cause an increment in the rate of growth of the larvae. The rate of growth would seem to be variable at different times of the year. All the larvae used in the above experiments were several weeks old when transferred to the experimental animals and yet when these lambs were slaughtered several weeks later small larvae were still present. On the other hand, it was seen from the routine examination of spring lambs in early summer that the parasites reach maturity in the course of 2 to 3 months.

It also appears from these studies that there may be at least two generations of the fly per year in this country. In view of this, it is surprising that Rodhain and Bequaert (4) report only one generation of adult flies per year in Europe. They also point out the cycle is more rapid in Africa and that sheep are probably infected at all times since they always found second as well as third-stage larvae in all the animals examined. In the present study 63% of the infected animals harboured larvae in two or more instars. Most of the animals which harboured one stage only were examined during the

winter months. This raises the question as to whether or not there are more than two generations of the fly per year in Africa.

Pupa

Pupae (Figs. 16, 17, and 18) were obtained by leaving mature larvae in dry sand at room temperature (approximately 20° C.). Metamorphosis was not successful in all pupae. The first larva which pupated was taken on April 5 and the last on August 12. The pupal period varied from 19 to 34 days with an average of 29 days in 12 specimens.

Effect of Larvae on Host

The larvae caused a definite irritation to young animals. This was especially apparent in those which were experimentally infected. They sneezed and rubbed their noses on the wall. The "snuffing" was continued throughout life but was not so marked after the first few days. About two weeks following infection there was a slight discharge of mucus from the nostril of each lamb. At the time of autopsy the sinuses of both lambs were partially filled with purulent material. The pus contained large numbers of eosinophile leucocytes. Large numbers of these cells were also apparent in the mucosa of one of the lambs. First-stage larvae were frequently found with red blood cells in their intestines, but, as it was only found in those that had been bathed in blood, it is not known whether they were ingested by the parasite from blood present in the nasal cavities following the slaughter of the animal.

Acknowledgments

The author wishes to thank Dr. H. B. Speakman, Director of the Foundation, for his advice and encouragement throughout this work; to express appreciation for the help and suggestions of Dr. S. Hadwen, whose knowledge of this and related parasites was brought to bear on the present problem; and to acknowledge the help of Dr. Hadwen and Mr. Norman Law in taking many of the photographs.

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CANADIAN JOURNAL OF RESEARCH

VOLUME 19

1941

SECTION D

Published by the
NATIONAL
RESEARCH COUNCIL
of CANADA

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

JANUARY, 1941

NUMBER 1

A STUDY OF THE MALE GENITALIA IN CALYPTRATE DIPTERA, BASED ON THE GENUS *GONIA* MEIGEN (DIPTERA: TACHINIDAE)¹

BY FRANK O. MORRISON²

Abstract

A review of the major papers on the 'genitalia' of calyptrate Diptera indicates the need of establishing the evident homologies within the group, studying homologies with other forms, studying the rotation of the parts known to occur in some forms, and studying the musculature.

Gonia has 'sternite V' of the abdomen, and all succeeding segments, modified for effecting copulation. The 'posterior angles of sternite V' are produced. A small apparent 'tergite VI' may be secondarily cut off from the previously fused segments VI and VII. A narrow 'tergite VII' articulates on the left side with an asymmetric 'sternite VI and VII'. Segment VIII has disappeared. 'Tergite IX', the genital tergite, is large. 'Sternite IX', a shovel-shaped ventral sclerite, is anterior to the 'phallus'. Segment X is represented by the 'lateral lobes of sternite X', the 'tenth sternal plate', and the 'anal forceps' and, in related forms, (not in *Gonia*), by the 'parapodial plates'.

The 'phallus' consists of a 'phallobase' with 'dorsal spine', a bell-shaped 'aedeagus', and protrusible 'penis'. The 'aedeagus' is formed of a dorsal 'paraphallus', lateral 'hypophalli', membranous 'ventralia', and apical membranous 'praeputium'. Anterior and posterior pairs of 'basal phallic appendages' surround the 'phallobase'. Internally a 'double apodeme' extends forward.

The term 'genitalia' is used to refer to the entire modified apex of the abdomen.

Introduction

The similarity in the structure of the apex of the abdomen, among higher Diptera, has long been recognized. However, the dissimilarity in this structure, from that among lower Diptera, or from that among other orders of insects, is none the less striking. For these reasons there has been no lack of studies on dipterous 'genitalia' but little agreement as to the segmental origin and general homologies of the parts.

An apparently varying number of apical abdominal segments have been modified for the purposes of effecting copulation. To this modified abdominal apex many terms have been applied. Thus Westhoff (87), Snodgrass (79), Feürborn (43), Petzold (71), and others refer to this apical portion of the abdomen as the "hypopygium". Metcalf (58) refers to it as "the post-

¹ Manuscript received in original form January 19, 1940, and as revised July 17, 1940.

Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que. Macdonald College Journal Series No. 147. Based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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abdomen". Patton (68) and Gibbins (45) argue that the term "terminalia" should be used and "genitalia" reserved for segment IX and its appendages; yet the term "genitalia" has been widely used in the literature to refer to the entire structure in question. The present writer has adopted the term 'genitalia' to refer to the entire apex of the abdomen and all associated structures.

Other morphological terms used in this paper are for the most part those employed by Snodgrass (81) or listed by La Torre-Bueno (54). Throughout the text single quotation marks enclose nomenclature employed by the writer. Table I lists the terms used by those writers whose works were reviewed. It is not always possible to be certain of the exact synonymy of such terms. Other writers have given similar comparative tables. In some cases these do not agree with the one presented here. This is due to differences in interpretation and possibly to mechanical errors in building tables. Thus Awati (27) lists his "alar projections of the theca" as synonymous with the "alar projections" of Hewitt (47), the "double apodeme" of Wesché (85), and the "great apodeme" of Lowne (55). An examination of these papers reveals that the "alar projections" of Hewitt and Awati are the 'posterior angles of sternite IX', which are directed posteriorly, whereas the "double apodeme" of Wesché and "great apodeme" of Lowne is the 'apodeme of the phallus' and is directed anteriorly. It is not perfectly clear from Awati's diagrams whether this is his "median process" or not. Again Awati appears to have misinterpreted Lowne with regard to segment VI, listing Lowne's segment VI? as equal to his segment V, while Lowne definitely states that sternite V is the last visible sternite and is deeply emarginate behind, thus identifying it with Awati's sternite V.

Similarly Petzold (71) appears to have confused Brül's (31) terms "lamina inferior" and "lamina superior" and Lowne's terms "hypophallus" and "paraphallus", wrongly synonymizing the first of the former with the second of the latter.

The appended list of references gives some idea of the extent of the accumulated literature on the copulatory apparatus or genitalia of the order Diptera alone. A review of even the major papers on the genitalia of calyptrate Diptera must in the interests of brevity be omitted from this publication; however, at least an indication of the varying interpretations by the different authors may be found in Table I. The numbers for papers cited in the list of references and those used for figures continue on from those appearing in an earlier paper by the writer on the taxonomy of *Gonia**.

The Male Genitalia of *Gonia*

GENERAL CONSIDERATIONS

It has not been possible, during the preparation of this paper, to investigate the matter of the rotation of the apical abdominal segments as described by Feüerborn (43) and Schröder (78). Keuchenius (52) indicates the ejacula-

* *Can. J. Research, D*, 18 : 336-362. 1940.

TABLE I
COMPARISON OF TERMS USED BY DIFFERENT WRITERS

—	Writer	(1)	(2)	(3)	(4)	(5)
1	Terms used in this paper	Sternite V	Tergite VI	Sternite VI and VII	Tergite VII	Tergite IX
2	Lowne, (55)	Lobes of sternite V Sternite V	Tergite VI	Epipleuron	Tergite VII	Tergite VIII Progenital tergum Tergite VIII
3	Brüel, (31)	Sternite V	Tergite VI	Chitining?	Tergite VII	Tergite VIII
4	Hewitt, (47)	Sternite V Primary forceps	Tergite VI	Sternite VI	Tergite VII	Tergite VIII
5	Wesché, (85)	Laminae superiores	—	—	—	—
6	Berlese, (28)	Sternite VI Primo forcipe	Tergite VIII	Sternite IX	Tergite IX	Tergite X
7	Newstead, (63)	Sternite VI	Tergite VII	—	Tergite VIII	—
8	Awati, (27)	Sternite V Accessory forceps	Tergite VI	Sternite VII	Tergite VIII	Tergite IX Genital tergite Tergite IX
9	Newell, (62)	Sternite V	Tergite VI and VII	Sternite VI and VII	Tergite VIII	Tergite VIII
10	Müller, (60, 61)	Sternite V	Tergite VI	—	—	Tergite IX
11	Feilerborn, (43)	Sternite VI	Tergite VII	Halbring	Tergite VIII	Tergite VIII
12	Petzold, (71)	Sternite V	Tergite VI	Sternite VI	Tergite VII	Tergite IX
13	Schräder, (78)	Sternite VI	Tergite VII	Basalring Extension of tergite 7 and 8 ?	Tergite VIII	Tergite IX
14	Cole, (33)	Sternite V	Tergite VI	—	Tergite VII and VIII	Tergite IX
15	Patton, (70)	Sternite V Lateral processes	Tergite VI	Sternite VI	Tergite VII	Tergite X
16	Townsend, (82)	Sternite V Anterior forceps	Tergite VI	Sternite VII	Tergite VII	Tergite IX
17	Snodgrass, (81)	Sternite V	Tergite VII	Sternite VIII	Tergite VIII	Tergite IX

TABLE I—Continued
COMPARISON OF TERMS USED BY DIFFERENT WRITERS—Continued

	Writer	(6)	(7)	(8)	(9)	(10)
—						
1	Terms used in this paper	Sternite IX	Tergite X	Sternite X	Lobes of sternite X	Anal cerci (segment X)
2	Lowie, (55)	Progenital sternum	Parapodial plates ?	Sternite IX	Valvae externae	Valvae internae
3	Brüel, (31)	Sternite VIII	Tergite IX	—	Valvula lateralis	Valvula medialis
4	Hewitt, (47)	Body of the penis	—	—	Sternite VII	Sternite VIII
5	Wesché, (85)	Part of the theca	—	—	Forceps inferior	Forceps superior
6	Berlese, (28)	—	Tergite XI	—	Forceps secundus	Mesocerci
7	Newstead, (63)	—	—	—	Sternite X	Tergite X
8	Awati, (27)	Vinculum	—	—	Editum	Superior claspers
9	Newell, (62)	Body of the penis	—	—	Inner claspers	Anal cerci
10	Müller, (60, 61)	Sternite X ?	—	—	Tergite X	Segment X
11	Feüerborn, (43)	Sternite VII	Tergite X	—	Seitenlappen ?	Mesocerci
12	Petzold, (71)	Vordere Gabelplatte	Tergite IX	Hintere Gabelplatte	Gonopöden	Tergite X
13	Schröder, (78)	Sternite 7 and 8.	Tergite X	—	Valvula lateralis	Forceps
14	Cole, (33)	Sternite VII	?	—	Valvula lateralis	Cerci
15	Patton, (70)	Sternite IX ?	—	—	Surstyli	Anal cerci
16	Townsend, (82)	Ninth tergo-sternum	—	—	Lobes of tergite IX	Paraprocts or anal cerci
17	Snodgrass, (81)	Vinculum	Tergite X	—	Ninth coxites	Segment XI
		Sternite IX	Tergite X	—	Lobes of Sternite X	Lobes of Tergite X
		Sternite IX	Tergite X	—	Lobes of Tergite IX	

TABLE I—*Continued*
COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Continued*

—	Writer	(11)	(12)	(13)	(14)	(15)
1	Terms used in this paper					
2	Lowne, (55)	Posterior angles of sternite IX Posterior angles	Anterior basal phallic appendages Anterior gonapophyses	Posterior basal phallic appendages Posterior gonapophyses	Anterior angles of tergite IX?	Processus longus Epipleural ridge
3	Brüel, (31)	Arm der Winkelhebels	Hakenfortsätze	Paramere	Gelenkfortsätze	Processus longus
4	Hewitt, (47)	Alar processes of the penis	—	—	—	—
5	Wesché, (85)	—	Palpus genitalium	Forceps interior	—	—
6	Berlese, (28)	—	Corpus penes	Ala corporis penis	—	—
7	Newstead, (63)	—	—	Inferior claspers?	—	—
8	Awati, (27)	Alar projections	Appendages of the vinculum? Lateral processes	Lateral processes?	—	—
9	Newell, (62)	—	—	Appendages of segment VIII	—	Appendages of segment XI? Processus longus
10	Müller, (60, 61)	—	—	—	Gelenkfortsätze	—
11	Feierborn, (43)	—	—	—	—	—
12	Petzold, (71)	—	Häkenfortsätze	Paramere	Processus brevis	Processus longus
13	Schräder, (78)	Hebelfortsätze	Häkenfortsätze	Paramere	Gelenkfortsätze der VIII T.	Processus longus
14	Cole, (33)	—	Genital palpi	Interior forceps	—	—
15	Patton, (70)	Lateral processes to coxites Vincular apophyses	Anterior part of paramere Anterior gonapophyses	Posterior part of paramere Posterior gonapophyses	Processes of tergite X	—
16	Townsend, (82)	—	—	—	—	—
17	Snodgrass, (81)	Arms of Sternite IX	Median plates?	Free pair of lobes embracing aedeagal base	—	Lateral bars to segment X?

TABLE I—Continued
COMPARISON OF TERMS USED BY DIFFERENT WRITERS—Continued

—	Writer	(16)	(17)	(18)	(19)	(20)	(21)
1	Terms used in this paper	Phallus	Phallic base	Spine	Aedeagus	Paraphallus	Hypophallus
2	Lowne, (55)	Penis	Bulb	Azygos spine	—	Paraphallus	Hypophallus and ventralia
3	Brüel, (31)	Penis	Pars basalis	Dorn	—	Lamina superior	Lamina inferior
4	Hewitt, (47)	Penis	Theca?	Superior apophysis	—	—	—
5	Wesché, (85)	—	Theca?	Spinus titillatorius	—	Paraphallus	Hypophallus
6	Berlese, (28)	—	—	Apophysis spinosa penis	—	—	—
7	Newstead, (63)	—	—	—	—	—	—
8	Awati, (27)	Theca	Theca	—	—	—	—
9	Newell, (62)	—	—	—	—	—	—
10	Müller, (60, 61)	Penis	Gründglied	Dorn	Mittelstuck	Appendages of segment IX Furca	End or horns Appendages of segment X Vomer
11	Feierborn, (43)	Penis	Theka	Dorn	—	—	—
12	Petzold, (71)	Penis	—	Dorn	—	—	—
13	Schröder, (78)	Penis	—	—	—	—	—
14	Cole, (33)	Aedeagus	—	Spinus titillatorius	—	Lamina superior	Lamina inferior
15	Patton, (70)	Phallosome	—	Posterior process	Body of phallosome	Paraphallus	Hypophallus
16	Townsend, (82)	Aedeagus	Phalotheca	Inferior apophysis of the phallobase	Phallus	Paraphallus	Hypophallus
17	Snodgrass, (81)	Phallus	Phallobase	—	Aedeagus	—	—

TABLE I—*Concluded*
COMPARISON OF TERMS USED BY DIFFERENT WRITERS—*Concluded*

	Writer	(22)	(23)	(24)	(25)	(26)
1	Terms used in this paper	Ventrals	Praeputium	Penis	Double apodeme	Sperm pump
2	Lowne, (55)	Included in the hypophallus	?	—	Giant apodemes	—
3	Brüel, (31)	Lamina lateralis	Distaler Rand der weichen Innenröhre	—	Tragplatte Sternite IX	Samenpritz
4	Hewitt, (47)	—	Glans ?	—	—	Ejaculatory apodeme
5	Wesché, (85)	Hypophallus	—	—	Double apodemes	Ejaculatory apodeme
6	Berlese, (28)	—	—	—	—	—
7	Newstead, (63)	—	—	—	—	—
8	Awati, (27)	—	—	—	—	—
9	Newell, (62)	—	—	—	Ejaculatory duct ?	—
10	Müller, (60, 61)	Seitenmembrane	Endstuck	—	—	—
11	Federborn, (43)	—	—	—	—	Sternite IX
12	Petzold, (71)	—	—	—	Penisstütze	Samenpumpe
13	Schröder, (78)	Lamina lateralis	—	—	Tragplatte Sternite VIII	Sternite IX
14	Cole, (33)	—	—	Penis	Double apodeme	—
15	Patton, (70)	—	—	—	Apodeme of the phallobase	—
16	Townsend, (82)	Ventrals	Praeputium	—	Vincular apodeme	—
17	Snodgrass, (81)	—	—	Penis	Apodeme	Ejaculatory bulb

tory duct in *Dexia canina* Fabr. as crossing over the alimentary canal but, though he studied several forms, he does not make a point of the winding of the duct about the canal. Tullock (83) says of *Stomoxys* that the duct "does not encircle the rectum as in *Glossina*." Minchin (59) found it encircling the canal in *Glossina*. It is thus not possible to conclude that the condition is general in Diptera and it is hoped when fresh material is available to investigate this matter in the species concerned here.

Neither has it been possible to do any detailed study of the musculature of the genitalia of *Gonia*. From what limited observations could be made on dried specimens, it appeared that the musculature closely resembled that found by Schröder (78) in *Calliphora erythrocephala*. No muscular connections between 'sternite IX' or the 'apodeme' and tergites V or VI were observed.

It has been possible, however, to thoroughly investigate the external morphology of the genitalia in *Gonia* and to compare it directly with that in many other forms.

STRUCTURE AND HOMOLOGIES OF THE PARTS

GROSS STRUCTURE

The 'genitalia' of *Gonia* consist of the sternite of segment V and the following segments VI to X inclusive. Segment XI has entirely disappeared or is included in X. Segment VI is mostly membranous and folded under segment V. Sometimes, however, a narrow dorsal plate, 'tergite VI' is present behind and beneath 'tergite V'. Ventrally, 'sternites VI and VII' are probably included in an asymmetric sclerite (*S. VI + VII*, Figs. 35, 36), just above the fifth sternite and closely united to it basally. On the left side this combined sternite articulates with 'tergite VII', which is a distinct narrow, dorsal, transverse plate, at rest partly hidden under 'tergite IX'. In the extended position a membranous area intervenes between segments VII and IX. It would seem that segment VIII has disappeared, a condition postulated by Awati (27) and Patton (68). Segment IX is the genital segment and is represented by a large tergite (*T. IX*, Fig. 35) and sternite (*S. IX*, Fig. 35) behind which the 'phallus' is borne. The anus is a long slit-like opening on a membranous area just posterior to 'tergite IX'. No sclerotic 'parapodial plates', such as occur lateral to the anus in some Tachinids (71) are present in *Gonia*. A pair of terminal 'anal forceps' (*A.C.*, Figs. 35, 36, 37) are fused into one structure. Lobes of sternite X (*Lb. S.X.*, Figs. 35, 36, 37) are present. Placed laterally, just anterior to the 'anal forceps' they articulate with the 'forceps' and with 'tergite IX', and by means of long sclerotic rods, 'processi longi' (*proc. l.*) with the 'tenth sternal plate' (*S. pl. X.*).

A pair of 'anterior basal phallic appendages' (*Ant. b. ph. app.*, Fig. 38) are fused to the ninth sternite and appear as outgrowths of it. Posterior to these is a second pair of appendages, the 'posterior basal phallic appendages' (*Post. b. ph. app.*). These are movably articulated. The phallus (*Ph. l.*, Fig. 37) is supported by 'sternite IX' between the extended 'posterior angles' of which it protrudes. Basally it articulates with the large internal 'double apodeme'

(*Ap.*) which extends anteriorly into the body. The 'phallus' itself consists of a phallobase (*Phlb.*), aedeagus (*aed.*), and internal 'penis'.

'Sternite V' (Fig. 36)

STRUCTURE OF THE PARTS

The fifth sternite in *Gonia* is the last externally visible one. It is considerably wider than the preceding sternites. Its lateral edges have folded over ventrally and the posterior lateral corners extended to form two prominent projections, 'the lobes of the fifth sternite' (*Lb. S. V.*, Fig. 36). The inner edges of the external lobes are folded back and, in the 'fissiforceps' group, curiously "escaloped". In all species the inner edges of the lobes bear many heavy black bristles. It is homologous with sternite V or sternite VI of other authors, according to the number of abdominal segments they recognized. The lobes have been variously termed "accessory forceps", "primary forceps", etc. (Table I).

'Tergite VI' (Fig. 35)

The sixth tergite in *Gonia brevisforceps* Tothill is represented by a wide membranous area posterior to 'tergite V'. Normally, when the 'genitalia' are in the resting position, this area is invaginated and only exposed narrowly on the sides, where the spiracles are present. The latter structures, then, just show anterior to 'tergite VII'. In some species a short, narrow, dorsal, sclerotized area has been observed. At rest it is half hidden beneath 'tergite VII'. A larger remnant of this tergite is present in *Cynomya cadaverina* (Fig. 32). A portion of 'tergite VI,' in this case, seems to be still adhering to the anterior edge of 'tergite VII', especially at the left end where the combined tergite articulates with 'sternite VI and VII'. In *Cynomya* these sclerites also articulate at their right ends. 'Tergite VI' is well developed in *Calliphora* (Fig. 31).

In *Drosophila melanogaster* segments six and seven have evidently fused completely. The segment posterior to five, and exactly similar to it in the structure of the tergite, has two spiracles on each side in the connective membrane just below it. A similar condition has been described by Awati (27) in *Lispa* where he considered it a further step in the evolution of the 'genitalia'.

Since in *Gonia* and other related forms the penultimate spiracle lies just on the edge of the apparent seventh tergite and the ultimate within it and, since in such forms as *Musca domestica* Linn. (Fig. 30), the apparent sixth and seventh tergites remain completely fused on the right side and overlap on the left where the apparent seventh crosses the sixth to articulate with 'sternite VI and VII', it would appear that these two tergites, once fused into one plate, became reduced, and secondarily separated in the interests of flexibility at that point. If this is the case their exact limits, i.e., whether or not the split came on the old line of fusion, is not certain and would explain the position of the spiracles and the apparent connection of what seems to be sternite VI with what seems to be tergite VII. As pointed out above, in *Cynomya cadaverina* Desv. there is evidence that this division is not along the old line of fusion as a portion of the original sixth tergite appears to be

united with seven along its anterior border and to form a lobe at the left end. This lobe is involved in the articulation with 'sternite VI and VII' which takes place on the suture.

'Sternite VI and VII' (S. VI + VII, Figs. 35, 36)

Immediately above the fifth sternite and connected closely to its base is an asymmetric sclerite which ends free in a somewhat expanded lobe in the membrane on the right side of the fly, but continues up on the left side to articulate with the seventh tergite. It appears to be homologous with a similar sclerite in other calyptrate flies (Figs. 30, 32).

The close association of this sclerite with the seventh tergite has led some investigators (27) to list it as sternite VII. This interpretation assumes that sternite VI has disappeared. Snodgrass (81) considers that the entire sixth segment has disappeared. The apparent seventh tergite then becomes the eighth and this asymmetric sternal plate, closely associated with it, sternite VIII. Still other writers questioned the sternal nature of this sclerite. Lowne (55) called it an "epipleurite" while Schröder (78) interpreted it as a prolongation of his tergites VII and VIII. The pupal study by the last named author threw little light on the origin of this plate though he observed it early in the development and determined that it took no part in the rotation of the genitalia.

Comparative morphology at the present time does not seem sufficient to finally settle this matter. However, if the complete union of segments VI and VII in *Drosophila*, and in *Lispa* (27) is noted, it may be seen how such a compound structure as 'sternite VI and VII' might develop and maintain connection with a secondarily separated apparent 'tergite VII' and still remain closely united to 'sternite V.' In *Cynomya* (Fig. 32) this asymmetric ventral sclerite maintains its articulation with the tergal plates on both sides though that on the left side is stronger and resembles the articulation in other forms. As previously pointed out, the detached sclerite labelled 'tergite VI' seems to be only a portion of this tergite. The rest remains attached to 'tergite VII'. Possibly it is all one sclerite which breaks easily when handled. Certainly the anterior lobe of 'tergite VII' on the left side seems to be a part of 'tergite VI' and it is just at the union of this lobe and 'tergite VII' that the asymmetric sternal plate articulates. For these reasons the sternite in question is here considered as 'sternite VI and VII'.

'Tergite VII' (Fig. 35)

The seventh tergite in *Gonia* is a narrow strip of sclerotized chitin, bounded anteriorly by connective membrane and normally lying almost against 'tergite V'. If a narrow apparent 'tergite VI' is present the two may be fused on the right side. Posteriorly 'tergite VII' is bounded and often overlapped by the large genital tergite, 'tergite IX'. A spiracle is present near each end. At the right end 'tergite VII' is free in the connective membrane or fused with the similarly free end of 'tergite VI'. The left extremity widens and forks,

one branch ending free and one articulating with the extremity of 'sternite VI and VII' just posterior to 'spiracle VI'.

In *Musca* (Fig. 30) the structure is similar, the left end being enlarged and crossing over the end of 'tergite VI' to meet 'sternite VI and VII', while the right ends of 'tergites VI and VII' are fused. In *Calliphora* (Fig. 31) 'tergite VII' is a prominent sclerite and according to Schröder is bound to the anterior end of 'sternite IX' by a strong muscle band. As previously noted this segment is fused with segment VI in *Drosophila* and *Lispa*.

Segment VIII

Segment VIII in *Gonia* has disappeared or is entirely membranous. There is no indication of the presence of any eighth pair of spiracles. Reichardt (77) found the sclerites of segment VIII very narrow and no spiracles present in some Asilids such as *Laphria flava* (Fig. 24). In this species also the hypopygium including segment VIII has rotated through 180 degrees necessitating a wide connective membrane in this area. In *Tabanus* (Fig. 26) segment VIII is also somewhat reduced. Again in the Dolichopodidae, Snodgrass (79) notes the tendency of segment VIII to lose its spiracles and to almost entirely disappear on the right side (Figs. 25, 25a). In the Syrphidae, according to Metcalf (58), segment VIII is large but possesses no spiracles and is represented by the tergite only, which is often asymmetrical (Figs. 27, 27a). In *Drosophila* (Fig. 29), there is a wide connective membrane and great flexibility between segments VII and IX, but no spiracles or sclerites. Because of this tendency of segment VIII to weaken and lose its spiracles and because of the wide connective membrane in this area in calyptrate flies, the assumptions of Awati (27), Patton (68), and Townsend (82), that segment VIII has disappeared seem more logical than the view of Snodgrass (81) that segment VI is missing or that of earlier workers that the anterior abdominal segment is missing. The view that the genital segment is VIII, which has lost its spiracles, seems untenable in the light of the known reasonably constant occurrence of the opening of the male duct behind 'sternite IX' in insects.

Petzold (71) noted the presence of a clear, transverse line across 'sternite IX' (his "Gabelplatte") and held it as evidence of the presence of two sternites, VII and VIII, in this structure (Fig. 34a). The line is evident in the homologous ventral plate in *Gonia* and in other forms examined. If it is indicative of the double origin of the plate, sternites VIII and IX must be involved as the exit duct is posterior to segment IX. However, in *Tabanus* (Fig. 26) that portion of 'sternite IX' which is beneath 'sternite VIII' is very similarly differentiated and has been designated the "apodeme of sternite IX" by Newell (62).

Schröder (78) found 'sternite IX' bound to 'tergites VIII and IX' by muscular connections while Brül (31) found muscles connecting the same plate and the 'apodeme' (his "Tragplatte") to 'tergites V and VI'. All in all, the evidence is conflicting and seems too slight to postulate the presence of 'sternite VIII' in the structure here called 'sternite IX' or in any other structure observed.

'Tergite IX' (Figs. 35, 36)

The ninth or genital tergite is a large, dorsally convex plate with a V-shaped emargination in its posterior edge. It is folded under laterally and its 'antero-lateral angles' extend as sclerotized rods (*a.a.T. IX*) which articulate with the outside of the extended 'posterior angles of sternite IX' (*Post. a.S. IX*) and are closely bound to these by connective membranes. The posterior angles articulate with processes on the 'anal forceps'. The sides are emarginate posteriorly, and from beneath them extend the 'lobes of sternite X', which articulate with them. Ventrally, connective membrane extends across between the folded lateral edges from the 'ninth sternite' posteriorly to the 'anal forceps'. In the connective membrane covering the V-shaped posterior emargination is found the long slit-like anal opening. The close connection of this sclerite with 'sternite IX', its position in relation to the anus, and its apparent similarity in form and function to 'tergite IX' in the Syrphids (Figs. 27, 27a), to 'tergite IX' in the Dolichopodidae (Fig. 25), seem to determine which sclerite it is. It seems improbable that it is tergite VIII (71), or tergite X, as suggested by Townsend (82).

'Sternite IX' (Figs. 35, 35)

'Sternite IX' is a slightly arcuate, shovel-shaped sclerite. Anteriorly it is rounded. The 'posterior' "corners" or 'angles' are produced caudad and upward into processes on either side of the 'phallus'. In *Gonia* these produced 'posterior angles of sternite IX' articulate laterally with the 'anterior angles of tergite IX' and apically with 'sternite X', the sternal plate posterior to the 'phallus'. In forms in which 'sternite X' is absent or fused to these arms (Fig. 33), they appear to articulate apically with the 'processi longi' of the 'lobes of sternite X'.

In *Gonia* (Fig. 38) the posterior portion of 'sternite IX' on either side of the 'phallus' is prolonged into two ventrally directed lobes, the 'anterior basal phallic lobes'. These are hollow, slightly curved, sickle-shaped, sclerotic projections bearing hairs on their apices and with their upper posterior angles prolonged to articulate with the phallic base. Petzold (71) found comparable lobes ("Häkenfortsätze") in *Ernestia rudis* but considered them as distinctly separate from 'sternite IX' (his "Gabelplatte"). He shows them articulating with two projections on 'sternite IX' and through a pair of very small sclerites at their bases with the 'phallus' and the 'anterior basal phallic lobes'. Whether these 'posterior basal phallic lobes' are a part of 'sternite IX', true appendages (62), or part of the 'phallus', is uncertain. They are present in many calyptrate forms but rudimentary in the housefly and absent in *Drosophila*. No homologous structures appear to be present in lower forms.

Distinctly articulated posteriorly with the above mentioned 'anterior basal phallic lobes' are a second pair of elongate, almost straight, tubular, sclerotic lobes, the 'posterior basal phallic lobes' (Fig. 38). Snodgrass (81) considers these structures in *Pollenia rudis* as phallic lobes rather than as true appendages. He states that they are not muscled. Schröder (78) found that

The ventral part of the aedeagus, the 'ventralia', is entirely membranous. The apical 'praeputium' is likewise membranous. The penis or true intro-mittent organ is a long narrow tubular structure equal in length to the aedeagus and concealed within it when at rest. This structure was observed once while material was being treated in cold potassium hydroxide but was so delicate that it was destroyed before a drawing could be prepared.

The 'Double Apodeme'

Extending upward and anteriorly from the 'phallobase' into the body, above 'sternite IX', is a sclerotic apodeme. Basally, where it articulates with the 'phallobase', this structure is narrow. It extends into segment V and broadens out transversely fan-like, with a vertical keel-shaped projection below. Many muscles are attached to this apodeme and these govern the movements of the phallus.

Lowne (55) termed the similar structure in *Calliphora* the "great apodemes". Other writers have homologized it with certain sternites (Table I) while Wesché (85) uses the terms "double rod"; "apodeme of the penis", and "double apodemes". The double nature of this structure is evident in some forms but in most calyptrate Diptera there is one fused structure. Newell (62) has shown *Tabanus* to have two entirely separate and distinct apodemes. Their homology with the structure in question is, however, uncertain. In *Musca* apodemes are entirely lacking and in *Drosophila* seem to be represented by a sclerotized portion of the ejaculatory duct. It does not seem that we are yet justified in postulating an origin, sternal or otherwise, for this structure.

As pointed out in the introduction, Awati (27) misinterpreted certain early authors in their uses of the terms for the "double apodeme".

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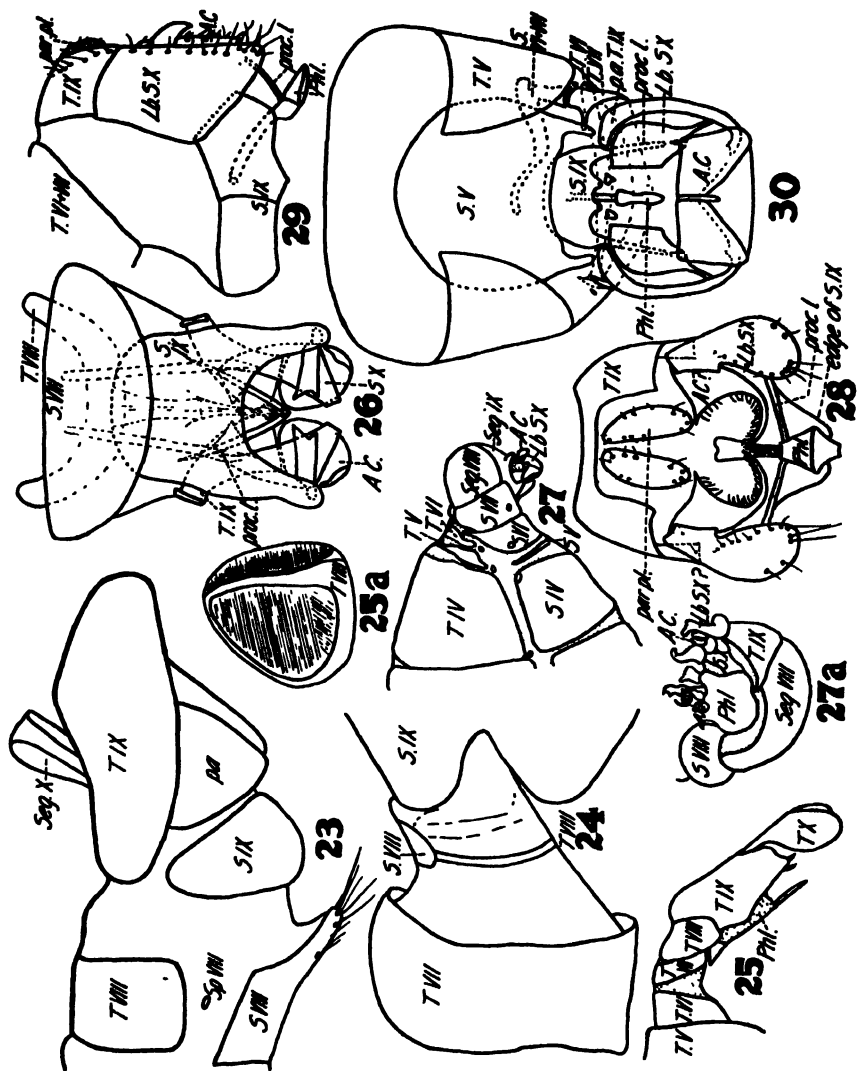


FIG. 23. Lateral view of the apex of the abdomen of *Machimus atricalpus* (Asilidae) (After Reichardt, 77).

FIG. 24. Lateral view of the apex of the abdomen of *Laphria flava* (Asilidae) (After Reichardt, 77). Note: hypopygium has revolved through 180 degrees; segment 8 is narrow.

FIG. 25a. The same as 25: segment 8 from the right hand side.

FIG. 26. Ventral view of the apex of the abdomen of *Tabanus* (Tabanidae).
FIG. 27. Tip of the abdomen of *Eristalis* (Syrphidae) (After Metcalf, 58). Numbering

FIG. 27a. Diagrammatic representation of the various parts represented in the male

genitalia of the Syrphidae, dextro-cephalic view (After Metcalf, 58). Numbering and labelling by the present writer.

FIG. 28. *Apex of the abdomen of Drosophila melanogaster, viewed from behind.*

FIG. 29. *The same as 28, lateral view.*

FIG. 30. *Ventral view of the apex of the abdomen of Musca domestica.*

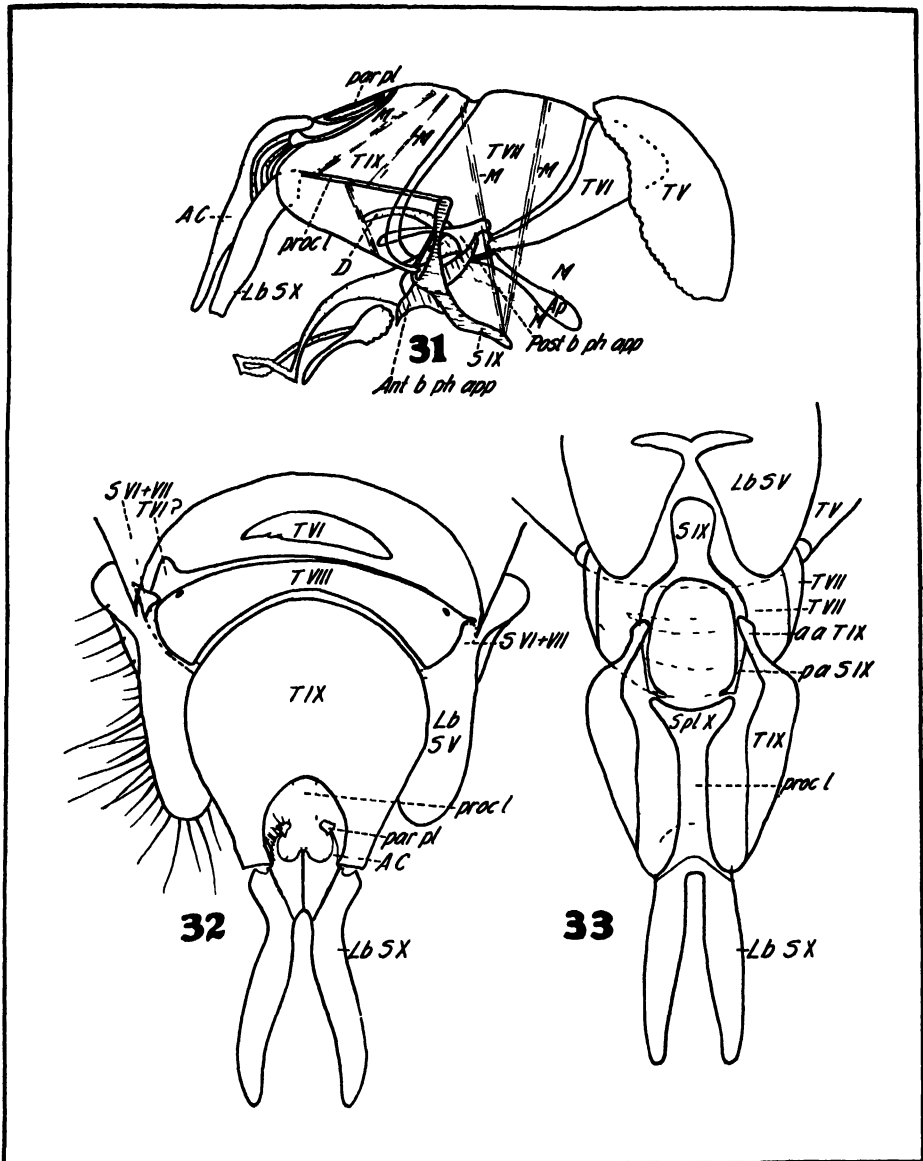


FIG. 31. Lateral diagram of the apex of the abdomen of *Calliphora erythrocephala* (After Schrader, 78). Numbering and labelling by the present writer.

FIG. 32. Dorsal view of the apex of the abdomen of *Cynomya cadaverina* Desv.

FIG. 33. The same as 32, ventral view, showing the fused, plate-like "processi longi". The 'phallus' and 'phallic' structures have been omitted.

they develop in the pupa from the second pair of genital lobes. This is of interest in view of the work done in tracing the development of genital appendages through immature forms in other insects. This work has been well summarized by Snodgrass (81) as follows:

"Studies on the development of the male genitalia in Trichoptera, Lepidoptera, and Hymenoptera have shown that the tubular phallic organ of these insects is formed during larval development by the union of a pair of genital lobes that grow out at the sides of the gonapophyses [Zander, (88, 89, 90); Singh Pruthi, (73, 74), Mehta (57)]. It is possible, therefore, that these larval phallic lobes of higher insects are homologues of the lateral phallogomeres of Mantidae and Blattidae. According to Zander, the primitive phallic lobes divide each into a median lobe and a lateral lobe, the two median lobes uniting to form the intromittent organ, while, in Trichoptera and Lepidoptera, the lateral lobes move to the sides and become articulated to the margins of the annulus of the ninth segment. We might, therefore, regard the median lobes as gonapophyses of the gonapophyses, and the lateral lobes (valvae or harpagones) as the styli. However, since it is claimed by Mehta that the lateral lobes in Lepidoptera arise separately from the median lobes, we cannot accept it as established that the gonapophyses of the male insect take any part in the formation of the intromittent organ, though there appears to be little doubt that they give rise to the styli or the movable claspers of the genital segment."

If Schröder's observations are accurate it would seem that these 'posterior basal phallic appendages' are the true appendages of segment IX and hence homologous with the large claspers of lower Diptera (Tipulidae, etc.), Trichoptera, and Lepidoptera (55). Certainly there do not seem to be any other structures present in calyptrate Diptera that represent the appendages of segment nine. Awati (27) and others have considered the parts here called lobes of 'sternite X' as the true appendages or at least as the coxites of segment nine. The evidence against this interpretation lies in the dorsal position, and dorsal and post-phallic articulation of the structures. Awati himself has called attention to this difficulty and solved it by merely stating that in his Syrphid type the appendages of the genital segment are dorsal and not ventral as in *Biblio*. Snodgrass considers that the appendages of segment IX are missing and that the structures in question, the 'posterior basal phallic appendages' are secondary outgrowths of the connective membrane about the base of the 'phallus'. He thus terms them phallic rather than 'periphallus' structures and his view is being adopted for the present.

Tergite X

'Parapodial plates'

There are no sclerites in the connective membrane surrounding the anus in *Gonia*. In *Drosophila* (Fig. 28) two small oval sclerites occur, posterior and lateral to the anus. These are here termed the 'parapodial' plates. In *Cynomya* (Fig. 32) they are represented by small triangular plates, on each

side of the anus, and in *Calliphora* (Fig. 31) by narrow lateral plates termed "tergite IX" by Br  l. Petzold (71) shows similar plates in some tachinids, in some cases free, in others fused with 'tergite IX'.

'Anal forceps' (Figs. 35, 36, 37)

The 'anal forceps' are the terminal structures in *Gonia*, when the genitalia are extended. At rest, however, they are folded beneath the abdomen with their apices in the genital pouch above the 'lobes of sternite V'. They are formed of two lateral sclerites, closely fastened together along the mid-dorsal line and divaricating only slightly at the apex. The lateral edges of the plates are rolled under and joined, at least basally, with connective membrane which is continuous with that extending ventrally across 'tergite IX'. Dorsally the forceps articulate with 'tergite IX' and laterally with the 'lobes of sternite X'. They vary greatly in width, depth, curvature, and setal vestiture, according to the species.

The anal forceps of *Pollenia rudis* and *Calliphora* are completely separate lateral lobes. In *Musca* (Fig. 30) they are flat and plate-like, but closely united with a very narrow sclerite inserted between them. Lowne (55) labelled them "sternite X". In *Cynomya* (Fig. 32) they are rudimentary, the small setae-bearing areas posterior to the 'parapodial plates' being the only indication of them. Similarly reduced 'anal forceps' are shown by Petzold (71) in certain Tachinidae. *Drosophila* (Figs. 28, 29) has two curved strips of slightly sclerotized chitin, below the parapodial plates, attached to the 'lobes of sternite X' and marked by strong setae on their edges. These appear to represent the 'anal forceps'. They are evident as posterior lobes of segment X in the Syrphids (Fig. 27a) and appear in some forms to be jointed. The folded under parts of 'tergite X' in *Tabanus* may represent these structures. The Dolichopodidae and Asilidae show no very similar structures but it seems probable that they are contained in the plates of the anus-bearing proctiger.

Snodgrass (81) and earlier workers find the anal forceps bound to 'tergite IX' by muscular connections which closely resemble the dorsal, intersegmental muscle bands.

'Lobes of sternite X' (Figs. 35, 36)

In *Gonia* there are present a pair of lateral lobes anterior to the 'anal forceps'. These are peculiarly contorted sclerites. Ventro-laterally, on each side of 'tergite IX' and somewhat beneath it, lies a somewhat triangular sclerite with the apex of the triangle curved ventrally and almost meeting its fellow from the other side in the membranous floor of the genital chamber. The base of the triangle is directed dorsally. Anteriorly it projects beneath 'tergite IX' and articulates with it, while posteriorly it articulates with the 'anal forceps'. Extending ventro-laterally from the anterior side of the triangle is a hollow, tubular, finger-like sclerite. The base of this projection is emarginate anteriorly. The projection is usually supplied with numerous setae apically. From the inside of the base a process, the 'processus longus', extends anteriorly in the floor of the genital chamber to articulate with the 'tenth sternal plate'

or directly with the posterior angles of 'sternite IX'. The origin of these curious triangular sclerites with their lobes and processes forms one of the major problems in the homologizing of the structures and explaining their segmental nature.

In *Tabanus* (Fig. 26) the tergite and sternite of segment X are conspicuous. Each is divided into lateral halves. The segment, the anus-bearing proctiger, extends anteriorly to sternum IX. The ventral plates of segment X articulate anteriorly with the 'ninth sternite', posterior to the phallic base.

In the Syrphidae (Fig. 27a) there are two styli-like lobes which articulate with 'tergite X' and 'sternite X'. *Drosophila* (Figs. 28, 29) has two large lobes extending on either side, outside of, but connected with, the 'anal forceps'. These lobes have definite 'processi longi' which connect them with the phallic base, posteriorly. In *Ernestia rudis* (Fig. 34) and in *Calliphora* the lobes resemble those in *Gonia* but are more strongly developed. Schröder (78) finds the 'processus longus' connected by muscles to 'tergite IX' but Snodgrass (81) considers these as unmusculated lobes. The 'lobes of sternite X' in *Cynomya* (Figs. 32, 33) have moved back to the place generally occupied by the 'anal forceps' and have united basally. The 'processi longi' have broadened and fused to form a definite 'tenth sternal plate' posterior to the 'phallus' and very similar to that present in some Syrphids. In *Musca* the lobes are flattened ventral plates mistaken for the seventh sternite by Hewitt (47).

The position of these lobes and their articulation, always posterior to the 'phallus', indicates their origin from the tenth segment and probably from the sternite of the same. They have frequently been termed the coxites of segment nine but their position relative to the 'phallus' makes this interpretation doubtful. Their shape, articulation, and lack of direct musculature suggests an origin other than from true appendages.

In view of the divided tenth sternite of *Tabanus*, the position of the lobes in question in *Gonia* and other calyptrate flies, and the formation of a ventral plate posterior to the 'phallus' by the fusion of the 'processi longi' in *Cynomya*, it is tentatively held here that these lobes have their origin in the tenth segment and are probably sternal. This is the view held by Townsend (82) though he states no reasons for it.

'Tenth sternal plate' (Figs. 33, 34, 36)

Posterior to the 'phallus' in *Gonia* (Fig. 36) lies a narrow, transverse, ventral plate with which the anterior ends of the 'processi longi' articulate. In *Pollenia rudis* this plate is turned on edge extending into the genital cavity and forming with the 'posterior angles of sternite IX' a basal shield for the 'phallus'. In *Musca* (Fig. 30) such a plate is absent or has split and the parts fused with the 'posterior angles of sternite IX'. The 'tenth sternal plate' in *Cynomya* has probably fused with the flattened and fused 'processi longi'. *Ernestia rudis* (Fig. 34) shows a condition similar to that in *Gonia*. Petzold (71) has designated this structure the "hintere Gabelplatte". Lowne (55) mentions a sternal plate posterior to the 'phallus' in *Calliphora* but does not name it.

In the Syrphidae (Fig. 27a) the 'tenth sternal plate' curiously resembles the composite plate in *Cynomya*. Such a plate, posterior to the 'phallus', might arise from a fusion of the extended 'posterior angles of sternite IX'. However, it seems more probable that it represents 'sternite X'.

The 'Phallus and Phallic Structures' (Fig. 38)

The 'anterior and posterior basal phallic appendages' have been dealt with previously. (See under 'Sternite IX'). There remains the 'phallus' proper and its 'double apodeme'.

The 'Phallus'

The theory of the segmental origin of the '*phallus*' in Diptera or any insect has been almost completely discarded. There is some evidence, according to Schröder (78) of its origin in Diptera from portions of appendages which have fused. However; as pointed out in the quotation from Snodgrass (see under 'Sternite IX') such an origin has not been proved the case in any insect. There is, however, a general basic plan according to which this structure in most calyptate Diptera may be interpreted.

In general the 'phallus' is an unpaired median structure. It is frequently referred to as the 'penis', but is a composite structure of more than the 'penis' proper which it ensheathes. Townsend (82) refers to it as the "aedeagus". Lowne (55) suggested a terminology. Brüel (31) gave a more complete terminology. Müller (60) took *Lucilia caesar* as a basis and developed a new set of terms for the structure in a "basic plan". Townsend (82) has chosen from earlier workers the names he prefers. Since the morphological homologies with structures in other insects are very doubtful as yet, any of these sets of terms serve the purpose of description equally well. The terms chosen for use here are those most frequently seen. Apparent synonymies have been listed in Table I, but since one term may in some cases cover parts of structures separately named by another worker exact synonymy is not always indicated.

In *Gonia* the 'phallobase' is tubular. Its anterior end articulates basally with the 'double apodeme' and is produced dorsally to articulate on each side with dorsal extensions of the 'anterior basal phallic appendages'. A membranous area occurs latero-dorsally in the 'phallobase'. Anteriorly it is produced into a dorsal 'spine'.

Closely attached to the 'phallobase' at right angles and with lateral apodemes extending into it, is the 'aedeagus'. It is narrow basally and expanded apically. A slightly sclerotized plate begins as the apodemes within the 'phallobase' and extends on the dorsal side of the 'aedeagus' where it divides forming a dorsal, spine-like structure, the 'paraphallus' or united 'paraphalli' ("Furca" of Müller), and two large lateral expansions which envelop the greater part of the 'aedeagus' laterally and may be narrowly united basally on the ventral surface, the 'hypophalli' ("Vomer" of Müller). These structures are sclerotized in successive small, scale-like patches, giving the surface a chequered appearance under high magnification.

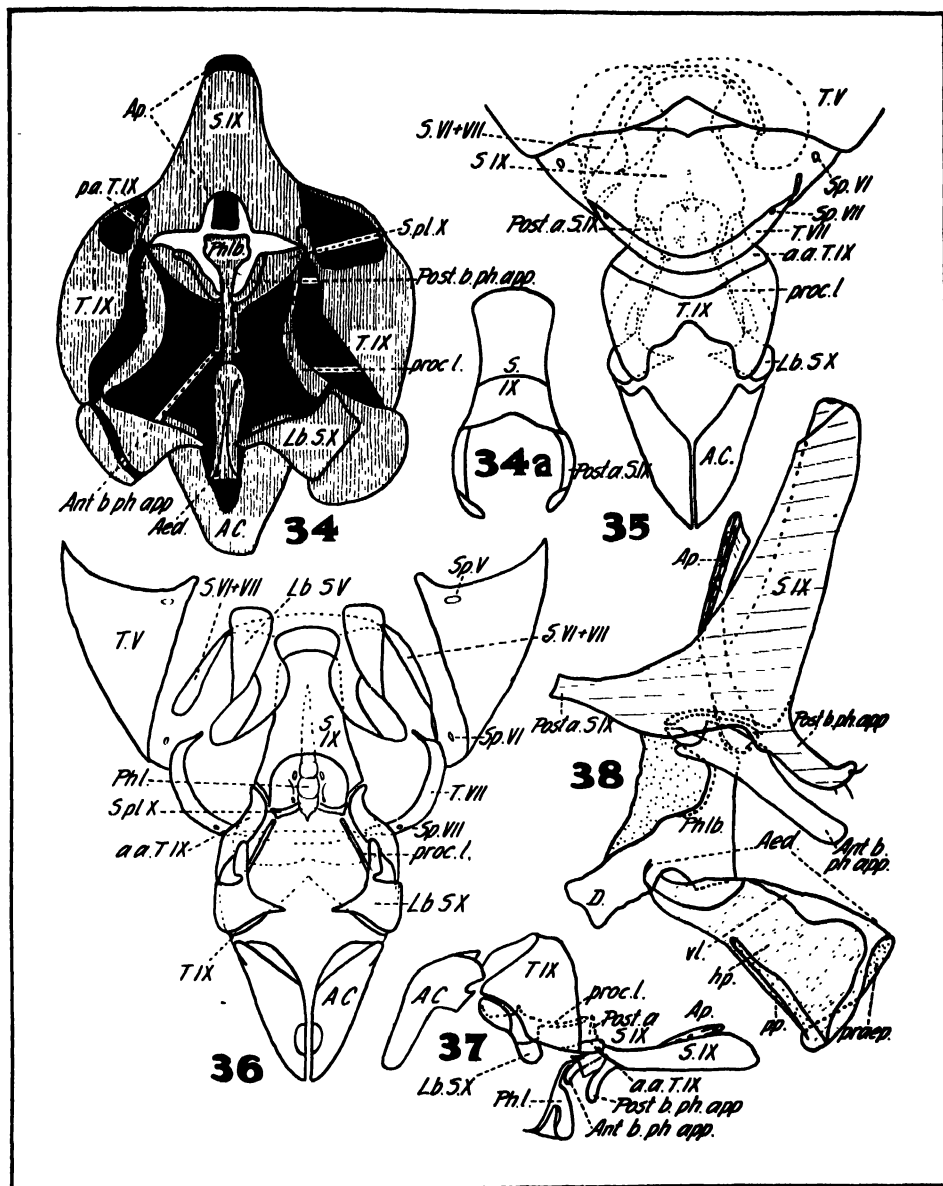


FIG. 34. Ventral view of the apex of the abdomen of *Ernestia rudis* (After Petzold, 71). Numbering and labelling by the present writer.

FIG. 34a. Ventral view of 'sternite IX' of *Ernestia rudis* (after Petzold, 71).

FIG. 35. Dorsal view of the apex of the abdomen of *Gonia breviforceps* Tothill.

FIG. 36. The same as 35, ventral view.

FIG. 37. The same as 35, lateral view.

FIG. 38. Lateral view of 'sternite IX' and the 'phallic' structures of *Gonia fissiforceps* Tothill.

CANADIAN WILTSHIRE BACON

XIV. SEASONAL VARIATIONS IN COLOUR AND COLOUR STABILITY¹

By C. A. WINKLER,² W. H. COOK,³ E. A. ROOKE,⁴ AND A. E. CHADDERTON⁴

Abstract

Measurements of the colour and colour stability of Wiltshire bacon, cured in a factory known to produce a generally satisfactory product, showed that there were small but significant differences in these properties from time to time, but there was no definite evidence to indicate that these differences could be attributed to systematic seasonal effects.

Introduction

Colour and colour stability are two important attributes of quality in bacon. Previous investigations (3, 4) on this subject have shown that, although Wiltshire sides cured at the same time in the same factory, may differ significantly in colour quality, the main source of variation generally lies in curing sides in different factories. This suggests that the colour quality of bacon depends primarily on the handling and curing practices followed in different establishments. There is also the possibility that colour quality may show seasonal variations, due either to the influence of environment on the animal prior to slaughter or to the effect of minor variations in the handling or curing conditions at certain stages where all the conditions are not subject to close control. The observations of certain practical operators suggested the existence of such seasonal variation in colour. The present study was undertaken to examine this possibility.

Material and Method

All the samples were obtained from a plant known to adhere closely to a practice that produced sides of consistently satisfactory colour quality. One side was taken at random from each weekly batch over a period of 20 months. After maturing at 32 to 35° F. for two weeks after removal from the curing tank, a portion, always taken from the same position in the prime back, was examined in the pale or unsmoked state.

The colour measurements were made with the colour comparators described in earlier publications. The first instrument (1) was used for the early measurements, and the improved form (2) during the remainder of the period. Owing to the higher "dark" constant of the first instrument, the readings so obtained were greater than those observed with the improved

¹ Manuscript received October 3, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Issued as paper No. 54 of the Canadian Committee on Storage and Transport of Food and N.R.C. No. 959.

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model. For this reason the results with the two instruments must be considered separately.

Estimates of colour stability were obtained from the change in colour during exposure for 24 hr. at 10° C. and 60 to 70% relative humidity. This relative humidity is lower than that employed in earlier measurements, and was chosen to include the effect of both drying and oxidation, comparable with that occurring in retail stores.

It has been shown in earlier papers (3, 4) that there is some variation between the colour and colour stability of different sides cured in the same tank at the same time. This inherent difference between sides must be considered in estimating the significance of differences that might otherwise be attributed to seasonal or time effects. Since it was impossible to provide samples from two sides, each week, it was assumed that the difference observed over a two-week period (three observations: beginning, middle, and end of period) would unlikely be seasonal, and consequently could be taken to represent the difference between sides. The observed differences were therefore analysed into portions attributable to within and between these two-weekly periods, taken as representing the difference between sides and seasonal effects respectively.

Results

The results of these analyses of variance appear in Table I. They show that on the average the differences between two-week periods, indicative of time effects, were usually significant for both the initial colour and colour stability measurements made with both instruments.

Figs. 1 and 2 were constructed from the results of the colour and colour stability measurements in an attempt to determine whether the significant differences, demonstrated between two-week periods, could be attributed to seasonal effects. Each point represents the mean of the three observations taken during each period. The central horizontal line indicates the general mean for all measurements made with the given instrument. The cross-hatched section on each side of the general mean indicates the necessary difference, computed from the variance within periods, between the individual points and the general mean.

It is evident from Fig. 1 that the initial colour during a given period seldom exceeded the necessary difference. The measurements made during June, 1938, with the original instrument were generally significantly below average, and increased to somewhat above average during August, 1938. Subsequent variations did not attain significance. Since the below- and above-average values observed in 1938 did not appear in 1939 when measurements were made with the new comparator there is no evidence to indicate that the difference between periods are attributable to systematic seasonal effects.

Fig. 2 shows the results of the colour stability measurements. The amount of light scattered by bacon generally decreases during exposure, and this was particularly true in these tests conducted under conditions that allowed

TABLE I
RESULTS OF ANALYSES OF VARIANCE ON COLOUR AND COLOUR STABILITY MEASUREMENTS

Source of variance	Degrees freedom	Mean square			Total brightness
		Red	Green	Blue	
<i>Initial colour of internal surface (original instrument)</i>					
Between two-week periods	17	40.28**	36.30**	35.68**	293.05**
Within two-week periods (Difference between sides)	36	8.29	5.24	2.84	42.40
<i>Initial colour of internal surface (new instrument)</i>					
Between periods	9	19.50*	6.90	4.04*	81.02*
Within periods	20	7.34	2.90	1.59	30.86
<i>Colour stability (original instrument)</i>					
Between periods	17	20.51**	3.89**	18.56**	99.94**
Within periods	36	3.84	1.02	2.58	15.32
<i>Colour stability (new instrument)</i>					
Between periods	9	3.48*	1.70**	0.69*	14.97**
Within periods	20	1.20	0.46	0.26	3.75

*Indicates 5% level of significance.

**Indicates 1% level of significance.

some drying to occur. In consequence, the initial value minus the final value is positive. This difference is used as the ordinate in the figure. Since the points are all greater than zero some darkening is indicated for all samples. A marked darkening, or instability, occurred during August, 1938, when the colours of the original samples were brighter than average. Otherwise none of the points show a significant departure from the average of all observations made with the original instrument. During August, 1939, the bacon, measured with the new instrument, showed greater stability than the average. This appears to be associated with the darker samples (Fig. 1) obtained at that time. From these results it appears that, although colour stability may be associated with the initial colour of the samples, there is no evidence of definite seasonal trends.

Discussion

Attempts to relate the significant departures from average colour quality to the use of frozen carcasses and to other minor changes in curing practice indicated that these were not the causative factors. Likewise the temperature records for the years 1938 and 1939 failed to show anything that would explain the variations. The significant departures from the average, therefore, remain unexplained.

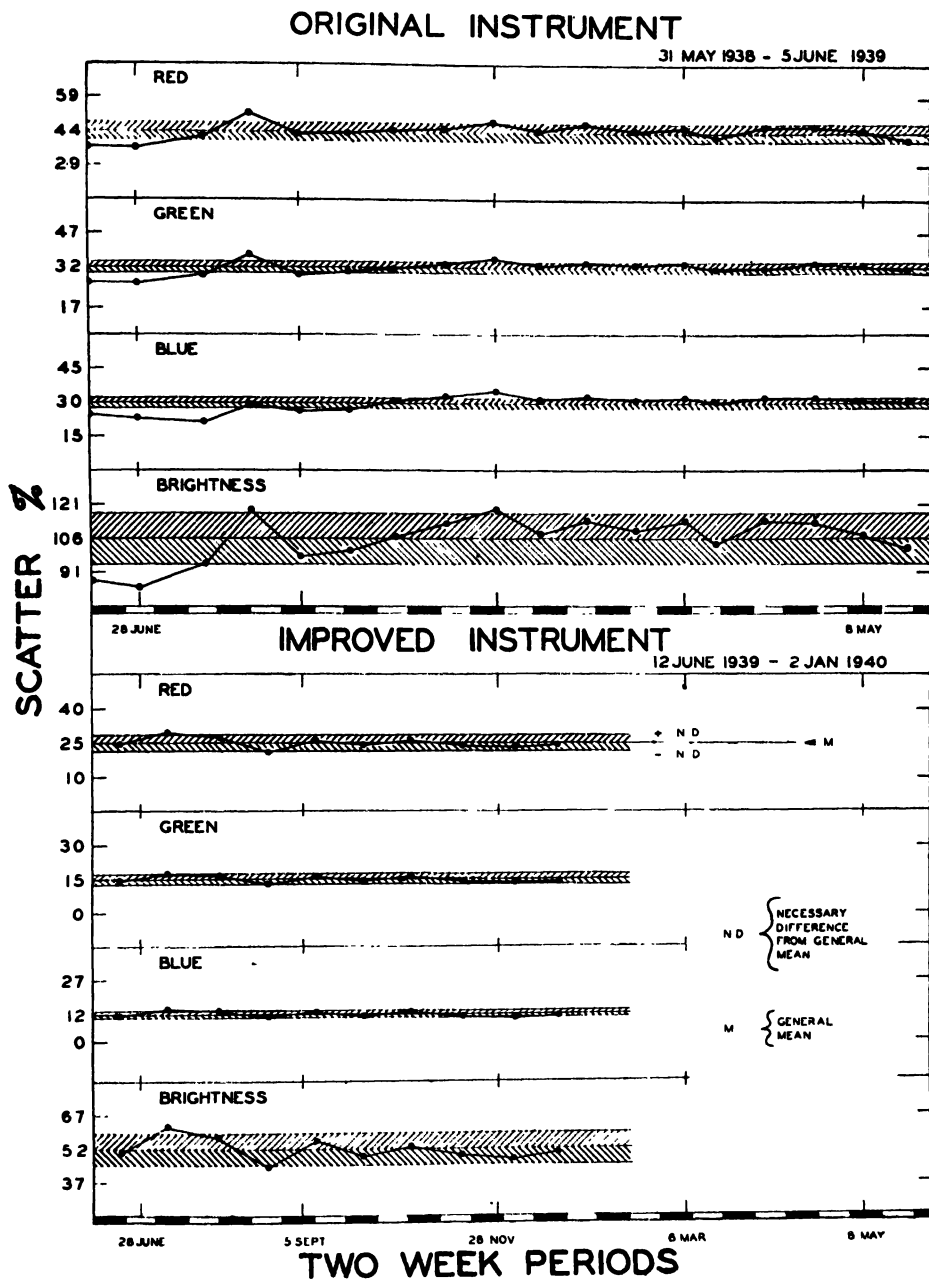


FIG. 1. Seasonal variations in colour scatter.

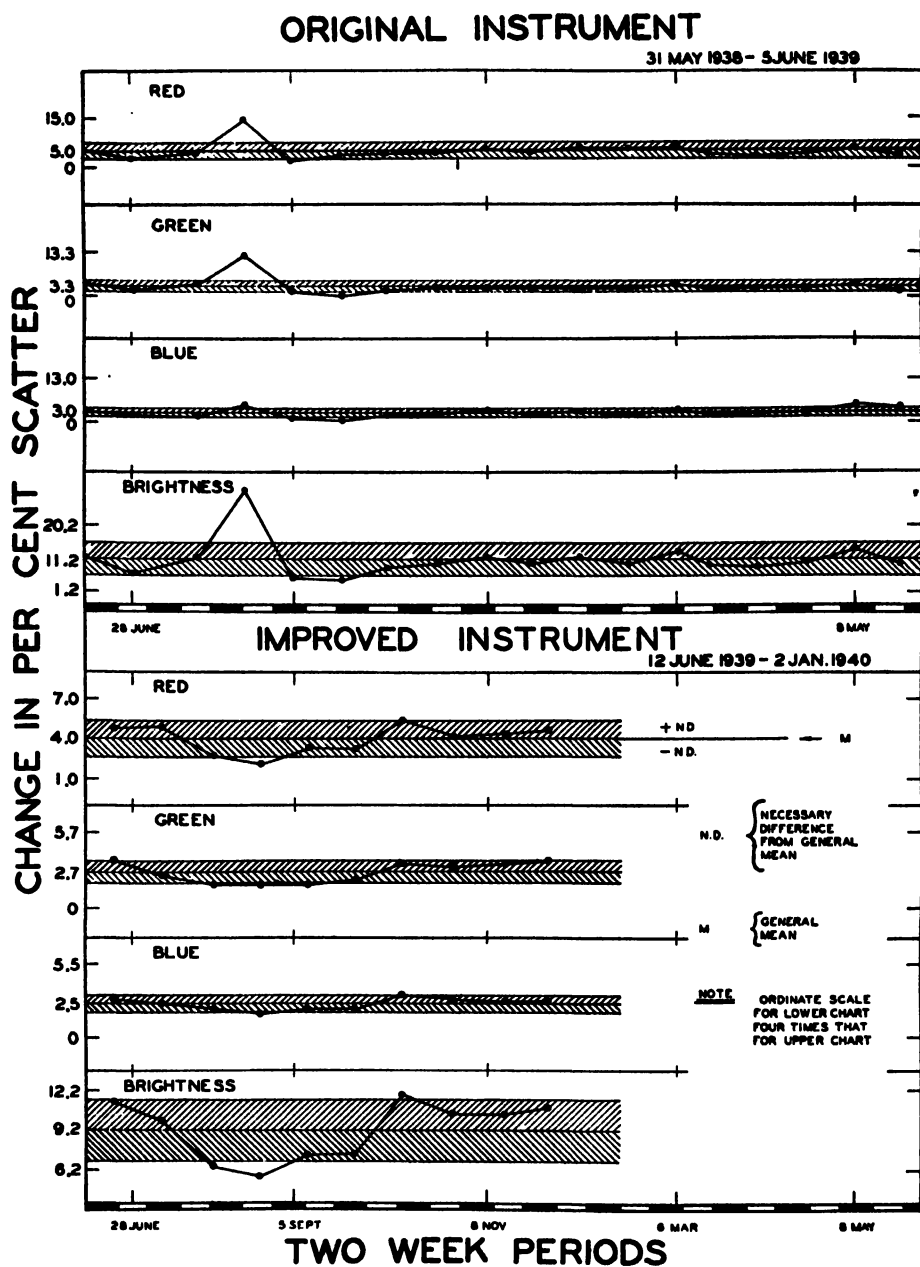


FIG. 2. Seasonal variations in colour stability.

It is entirely probable that the curing and handling practices followed in certain factories may enhance possible seasonal variations in colour quality. The results of the present investigation, however, indicate that practices could be adopted that would render the product relatively independent of these effects, but the nature of the detrimental practices, if any, must await the results of future investigations.

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A CRITICAL STUDY OF STAFFORD'S REPORT ON "TREMATODES OF CANADIAN FISHES" BASED ON HIS TREMATODE COLLECTION¹

BY MAX J. MILLER²

Abstract

The internal trematodes from fish in the late Dr. Stafford's slide collection have been restudied and described. *Gasterostomum armatum* of Stafford is identified with *Prosorhynchus squamatus* Ohdner, 1905, and *Crepidostomum laureatum* with *C. cooperi* Hopkins, 1932. *Homalometron pallidum* Stafford, 1904, is redescribed and it is suggested that the genus *Homalometron* Stafford, 1904, is synonymous with *Lepocreadium* Stossich, 1904. *Neophasis pusilla* Stafford, 1904, and *Stenakron vetustum* Stafford, 1904, are redescribed and assigned to the family *Allocreadiidae*. *Hemiurus appendiculatus* of Stafford, is demonstrated to represent *H. levinsoni* Ohdner, 1905, and *Brachyphallus crenatus* Rudolphi, 1802, while *Felodistomum incisum* of Stafford represents the species *F. fellis* Olsson, 1868, and *F. agnotum* Nicoll, 1909. Species of the genera *Azygia* and *Olodistomum* are reidentified. *Protenteron diaphanum* Stafford, 1904, is redescribed and the species is referred to the genus *Cryptogonimus* Osborn, 1903, the new combination being *Cryptogonimus diaphanus*.

Introduction

The late Dr. Stafford's report (19) on the "Trematodes of Canadian Fishes" is well known to all who have made studies on the internal trematodes of fish. In this report he created 18 new genera and 14 new species; unfortunately in most cases the descriptions were meagre and accompanied by no supporting illustrations. Later workers have experienced great difficulty in trying to identify his forms and considerable confusion has resulted from attempts to assign the proper nomenclature for the species he described as new, as well as for the generic names he founded. It has been suggested that certain of his genera are not valid and that some of his identifications are not accurate. It is appreciated, of course, that owing to the unsettled state of trematode systematics at the time of his study and the difficulty he must have experienced in obtaining necessary literature, certain of his errors in identification could not be avoided. However, it must be admitted that the criticism of his contemporaries, in stating that he founded genera and species with far too inadequate a description, is valid.

The opportunity to study Dr. Stafford's slide collection has recently presented itself; although a few of the species he described are not present in the collection, the majority are present. Most are in a fair state of preservation, and some have retained their stain very well. A difficulty was encountered in attempting to connect the trematodes with his descriptions; in some cases the name of the parasite is not given, the slides being labelled only with

¹ Manuscript received August 14, 1940.

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Quebec, with financial assistance from the National Research Council of Canada.

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the name of the host. In those cases where the names are given the genus is often represented by the letter, D. referring no doubt to *Distoma*. However, by comparing the morphology of the parasites with his description and taking into consideration the hosts and location of the parasites in the hosts, practically all his specimens could be assigned to their proper positions.

In the present report the species will be presented in systematic order with no attempt to follow the order in which they were presented by Stafford. In those cases where Stafford's nomenclature is wrong, his names are presented in parentheses underneath the proper name.

Description of Parasites

FAMILY BUCHEPHALIDAE

Prosorhynchus squamatus Ohdner, 1905

(Fig. 1)

(*Gasterostomum armatum*, of Stafford)

HOST: *Brosmius brosme* (cusk), from the intestine?

Ohdner (13) pointed out that *Gasterostomum armatum* Molin, 1861, and *Gasterostomum armatum* of Olsson, 1868, and of Levinsen, 1881, represent different species. As *G. armatum* Molin, 1861, is synonymous with *Monostomum crucibulum* Rudolphi, 1819, Ohdner created the genus and species *Prosorhynchus squamatus* for the species represented by *G. armatum* of Levinsen and Olsson. He distinguished *Prosorhynchus* from *Bucephalus* (*Gasterostomum*) by its rostellum-like anterior sucker, the arc-like position of the vitellaria, and the character of the male genitalia.

The collection contains a single specimen from the cusk. It measures 2 mm. long by 0.8 mm. wide. The body is egg-shaped and covered with fine, closely set spines. The anterior sucker is rostellum-like in shape and measures 0.2 mm. by 0.18 mm. The mouth opening is just posterior to the middle of the body, and the ventral sucker surrounding it measures 0.16 mm. in diameter. The testes are slightly broader than long, situated in the third quarter of the body in tandem arrangement. The ovary is subspherical, situated just above the anterior testis and partially overlapping it. It is somewhat larger than the testes. The uterus is in the posterior half of the body and the genital pore near the posterior tip. The vitellaria form an arc midway between the rostellum and the mouth opening. The cirrus sac lies on the left side of the body; it is very large, measuring 0.64 by 0.3 mm. The eggs measure 0.032 by 0.041 mm. by 0.021 to 0.025 mm.

The specimen in the collection differs from the material described by Ohdner in the somewhat larger egg size, the larger cirrus sac, and the fact that the ovary is larger than the testes. While these characters may constitute the basis for the establishment of a new species the fact that only a single, considerably flattened specimen is available for study does not exclude the possibility that the differences may merely be variations within a species.

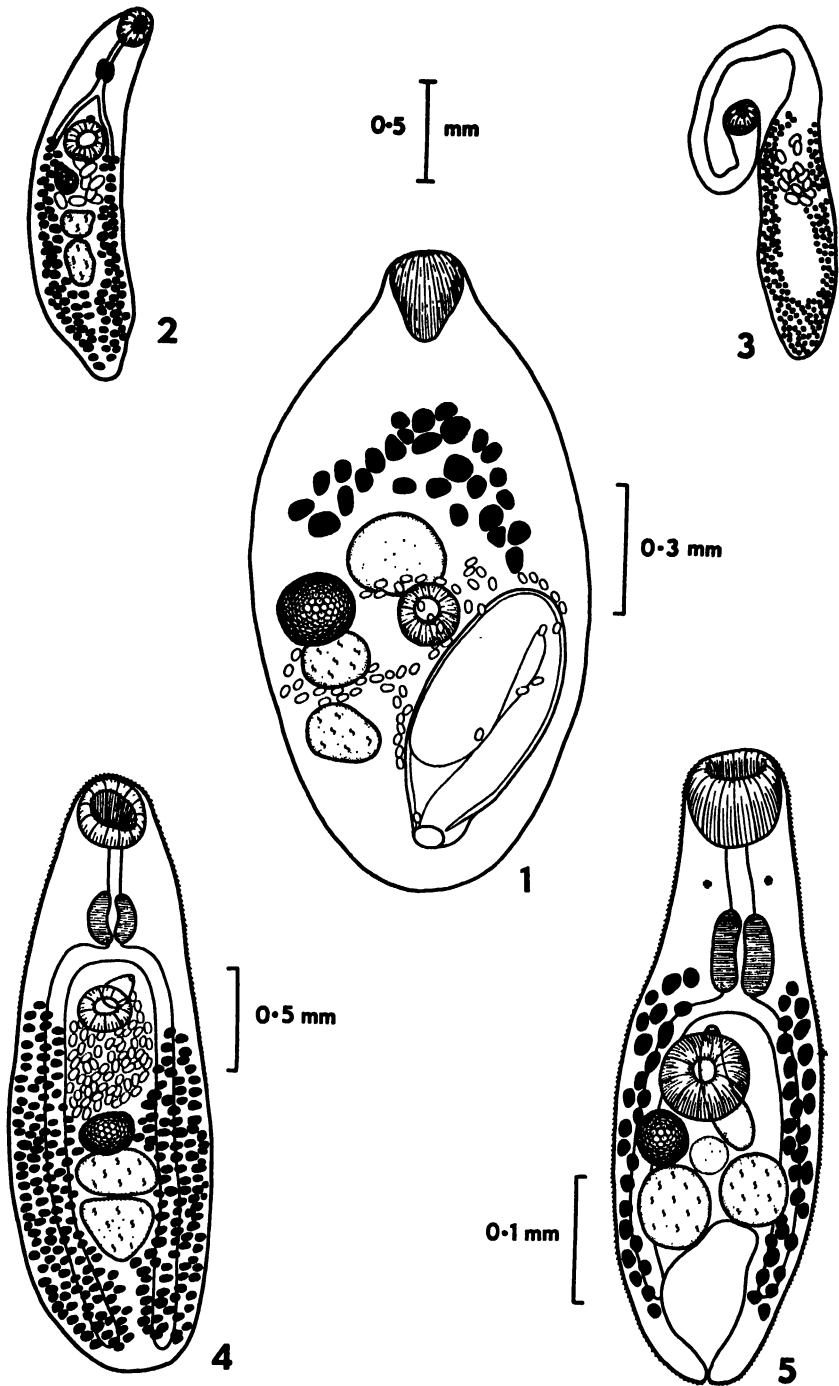


FIG. 1. *Prosorhynchus squamatus*, ventral view. FIG. 2. *Homalometron pallidum*, ventral view. FIG. 3. *Stenocollum fragile*. FIG. 4. *Lepidapedon rashion*, ventral view. FIG. 5. *Neophasis pusilla*, ventral view.

FAMILY ALLOCREADIIDAE

Crepidostomum cooperi Hopkins, 1932

(Fig. 6)

(*Crepidostomum laureatum*, of Stafford)Host: *Salvelinus fontinalis* (speckled trout), from the intestine.

The collection contains several well preserved specimens of this species ranging from 1.1 to 1.6 mm. in length. Hopkins (1) has pointed out that *Crepidostomum laureatum* Zeder, 1800, is synonymous with *C. farionis* Müller, 1784, which is quite distinct from the specimen in Stafford's collection.

Genus *Lepidapedon* Stafford, 1904*Lepidapedon rachion* (Cobbold, 1858)

(Fig. 4)

Host: *Melanogrammus aeglefinus* (haddock), from the intestine.

Stafford created the new genus *Lepidapedon* for *Distomum rachion* of Cobbold, but gave no description of it. Ohdner (13) gave a comprehensive description of this species and proposed the new genus *Lepodora*. However, Stafford's name holds priority and must be considered as correct.

The collection contains four specimens ranging from 1.5 to 3.1 mm. long by 0.4 to 1.0 mm. wide. None measure 5.0 mm. in length, the measurement given by Stafford for this species. The specimens are broadest in the third quarter of the body length, and narrow somewhat to the posterior end and more markedly to the anterior tip. The cuticula is covered with numerous spines which are more conspicuous at the anterior end of the body. The oral sucker is larger than the acetabulum and measures from 0.2 to 0.38 mm. in diameter. There is a long pre-pharynx and a conspicuous pharynx about the same size, being from 0.14 to 0.26 mm. in length. The oesophagus is very short and divides almost immediately into the intestinal caeca which extend to the posterior tip of the body. The acetabulum is situated in the second quarter of the body and measures from 0.13 to 0.35 mm. in diameter. The testes and ovary are roughly ovate in outline with the long axis horizontal. They are in tandem arrangement in the third quarter of the body with the ovary immediately anterior to the anterior testis. The uterus lies between the ovary and genital pore, which is situated midway between the intestinal bifurcation and the acetabulum, to the left of the mid-line. The broad cirrus sac overlaps the acetabulum for about half its diameter. The vitellarian follicles extend laterally from the acetabulum to the posterior tip of the body and fill in the post-testicular body space. The eggs measure 0.070 to 0.078 mm. by 0.042 mm.

Homalometron pallidum Stafford, 1904

(Fig. 2)

Host: *Fundulus heteroclitus* (killifish), from the intestine.

The generic and specific names of this parasite were proposed by Stafford who gave no description but referred to the description of Linton (4, 5).

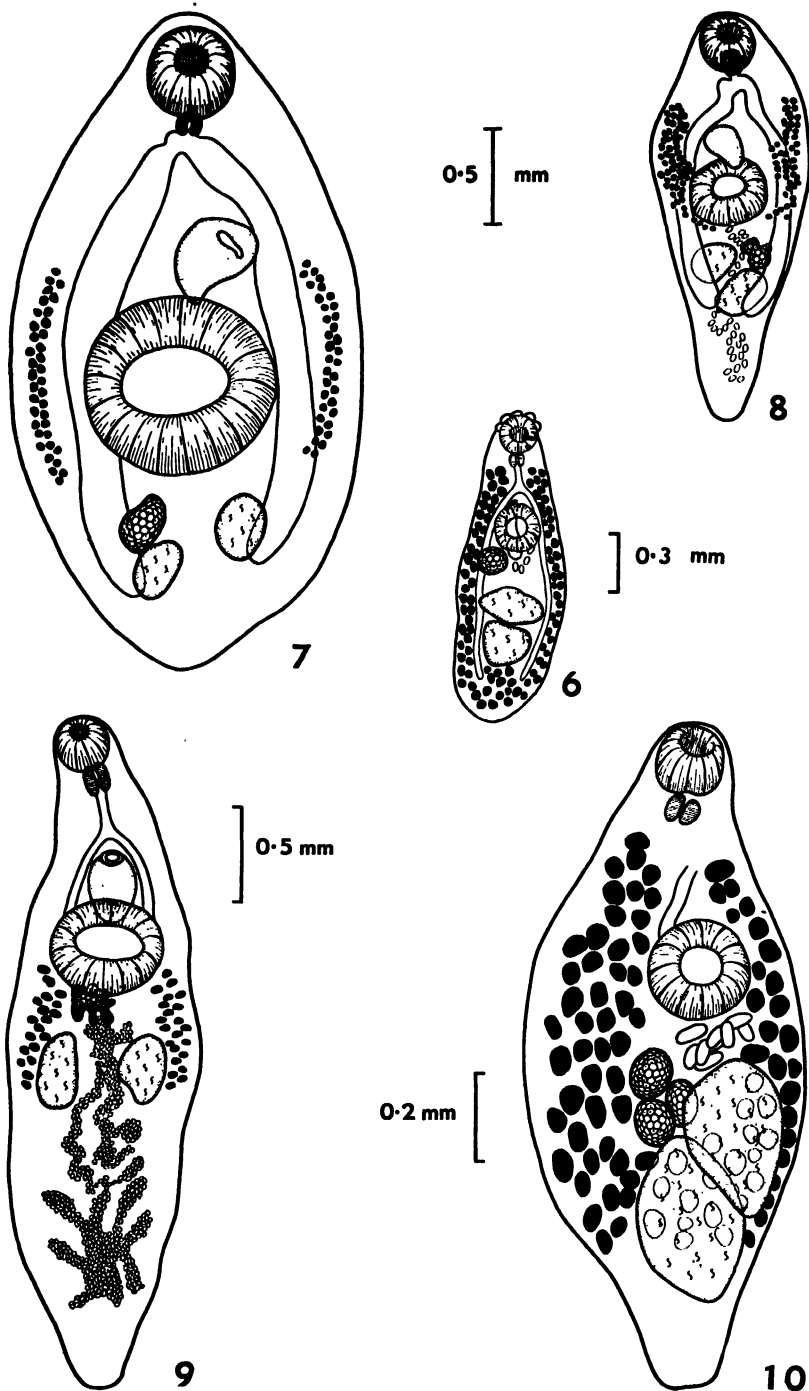


FIG. 6. *Crepidostomum cooperi*, ventral view. FIG. 7. *Fellodistomum fellis*, ventral view.
 FIG. 8. *Fellodistomum agnotum*, dorsal view. FIG. 9. *Steringophora furciger*, ventral view.
 FIG. 10. *Stenakron vetustum*, ventral view.

Looss (7) considers *Homalometron* synonymous with *Lepocreadium* (Stossich, 1903). Manter (10) considers the two genera closely related but differentiable on the basis of the position of the genital pore, which he states is median in *Homalometron* and to the left of the mid-line in *Lepocreadium*, and the presence of a cirrus pouch in the latter genus and its absence in the former. However, Linton (4) states that the genital pore is median in *Lepocreadium trulla*, and his figures of this species show the pore to be in a median position. He states that certain specimens, which he assigns to the species *L. levinseni*, but which he suspects may belong to another species, do not have a cirrus pouch. It thus seems not unlikely that *Homalometron* may be a synonym of *Lepocreadium* Stossich, 1904. However, until all species are carefully compared it seems wiser to consider them as distinct genera for the time being.

The specimens in the collection are small, narrow forms measuring 1.76 to 1.94 mm. by 0.040 to 0.048 mm. They are broadest in the region posterior to the middle and taper somewhat posteriorly and more markedly to the anterior tip. The cuticula shows evidence of being spinose although most of the spines have been lost. The oral sucker is terminal and subspherical in outline; it leads by an obvious pre-pharynx to a conspicuous pharynx of about the same length. The short oesophagus divides into the intestinal caeca which extend to near the posterior tip of the body. The acetabulum is situated about one-third of the body length from the anterior tip. It is somewhat larger than the oral sucker. The testes are in tandem arrangement in the second half of the body. They are subspherical to oval in shape and may be somewhat irregular in outline. The ovary is anterior to the anterior testis and is displaced slightly to the right. The uterus leads back to the anterior margin of the anterior testis rather than forward to the genital pore situated immediately anterior to the acetabulum on the mid-line. There is no cirrus pouch. The vitellaria extend laterally from the posterior half of the acetabulum to the posterior tip of the body, and fill in the post-testicular space. An average specimen shows the following measurements: length 1.9 mm., width 0.5 mm., oral sucker 0.18 by 0.15 mm., acetabulum 0.23 mm. in diameter, pharynx 0.095 mm. long, eggs 0.092 to 0.11 mm. by 0.54 mm.

Neophasis pusilla Stafford, 1904

(Fig. 5)

Host: *Annarhichas lupus* (wolf fish), from the urinary bladder.

Stafford created the new genus and species *Neophasis pusilla* for a small trematode he obtained from the urinary bladder of the wolf fish. Lebour (3) makes brief mention and gives diagrams of a trematode from the stomach of the wolf fish. In a later report (1909) she refers it to the genus *Acanthopsolus* of Ohdner (13) under the name *A. lageniformis*. Ohdner (14) considers *A. lageniformis* a synonym of *Neophasis pusilla* but he suppresses the genus *Neophasis* in favour of *Acanthopsolus* and retains Lebour's name of *A. lageniformis* for this parasite. Although the author has not seen Lebour's later report (1909), her diagrams of this parasite in the earlier publication (3)

suggest it to be a different species from *N. pusilla*. In Lebour's species the genital pore is situated on a level with the pharynx on the left side of the body, and the cirrus pouch lies wholly in front of the acetabulum, whereas Stafford's specimens have the genital pore immediately anterior to the acetabulum on the mid-line, and the cirrus pouch extends some distance below the posterior margin of the acetabulum. The author does not believe Ohdner (14) is justified in suppressing the genus name *Neophasis*, as the characters presented by Stafford are sufficient for the recognition of the genus. *Acanthopsolus* is therefore considered a synonym of *Neophasis* which contains the following three species: *N. pusilla* (Stafford, 1904) as the type species, *N. lageniformis* (Lebour, 1909), and *N. oculatus* Ohdner, 1905.

Poche (17), Fuhrmann (2), and more recently Ward (22) all place the genus *Neophasis* (*Acanthopsolus*) in the family Acanthocolpidae, although Ward (22, pp. 509-521) states that it disagrees with the family concept, and erects the subfamily Acanthopsolinae for its reception. However, in general morphology this genus shows a much closer relationship to the Allocreadiidae than it does to the Acanthocolpidae. *Neophasis* is accordingly considered by the writer as belonging to the family Allocreadiidae and tentatively placed in the subfamily Lepocreadiinae.

The material in Stafford's collection consists of six specimens, the largest of which measures 0.60 mm. in length. As Stafford has stated they are shaped like an Indian club with a rounded posterior end and a square cut anterior tip, which ends in the large oral sucker. The entire cuticula is covered with prominent closely set spines. The terminal oral sucker is subspherical. It leads by an elongate pre-pharynx to a large pharynx that measures somewhat less than the oral sucker. There is no apparent oesophagus, and the pharynx leads directly into the intestinal caeca which extend to within a short distance of the posterior tip of the body. The acetabulum is spherical, slightly smaller than the oral sucker, and is situated about half-way down the body length. There is a prominent eyespot on either side of the pre-pharynx. The testes are oval to subspherical in outline, side by side in a slightly oblique arrangement; the left testis is the more anterior. The ovary is subspherical, and is situated between the right testis and the acetabulum. The uterus extends from the anterior margin of the testes to the genital pore, which is situated on the mid-line immediately anterior to the acetabulum. The cirrus pouch extends posterior to the acetabulum, almost to the level of the testes. The vitellaria extend laterally from the pharynx to near the posterior tip. The excretory bladder is small and sac-shaped. The exact size of the eggs could not be determined as they were badly collapsed, but they appear to be quite large and to measure roughly about 0.08 mm. in length. Measurements of an average specimen are as follows: length 0.52 mm., width 0.18 mm., oral sucker 0.076 mm. in diameter, ventral sucker 0.071 mm. in diameter, pharynx 0.067 mm. long by 0.054 mm. wide, right testis 0.070 mm. in diameter, left testis 0.063 mm. in diameter, ovary 0.044 mm. in diameter.

Genus *Podocotyle* Stiles and Hassall, 1898

Synonym: *Sinistroporus* Stafford, 1904

Podocotyle atoman Rudolphi, 1802

(*Sinistroporus simplex* in part, of Stafford)

(Fig. 14)

Hosts: *Cottus scorpius* (sculpin)

Scomber scombrus (mackerel)

Sebastes marinus (rose fish), from the intestine.

Stafford created the genus *Sinistroporus* for *Distomum simplex* Rudolphi. Ohdner (13) has pointed out that *D. simplex* is a synonym of both *D. angulatum* and *D. atoman*. He further states that *D. angulatum* which was made the type species of the genus *Podocotyle* by Stiles and Hassall (20) cannot be retained because it was inadequately described, and because the type material has been lost. Thus *P. atoman* becomes the proper name of this species and the type of the genus.

The collection contains 12 specimens of this species, some in a good state of preservation. They agree quite well with the detailed description of Ohdner (13). Specimens range from 1.45 to 3.81 mm. in length.

Podocotyle reflexa Creplin, 1825

(Fig. 11)

(*Sinistroporus productus* and *S. simplex* in part, of Stafford)

Hosts: *Hemitripteris americanus* (sea raven)

Urophycis tenuis (hake), from the intestine.

Stafford included two species in his genus *Sinistroporus*: *S. simplex* and *S. productus*. He described *S. productus* which he named as a new species, as follows: "6.93 \times 0.60, long and narrow. Testes elliptical and some distance apart in the longitudinal axis of the worm." This is his entire description, and while it is certainly not sufficiently detailed to differentiate a new species there is little doubt that Stafford actually was dealing with a species different from his *P. simplex*. None of the slides in the collection are labelled *S. productus*. However, he gives the host of *S. productus* as *Hemitripteris americanus*, and all the specimens of *Podocotyle* that he has in the collection from *H. americanus* prove upon examination to be *P. reflexa*.

Ohdner (13) separates *P. reflexa* and *P. olssoni* by the proportion of the body width to length, the proportion of the length of the oesophagus to that of the pharynx, the extent of the cirrus sac, and the outline of the cross-section of the body. According to these characters the specimens from the sea raven agree with the species description of *P. reflexa* as the proportion of the body width to length ranges from 1 : 9 to 1 : 12, and the cirrus sac does not reach to nearly half the distance between the acetabulum and the ovary. The specimen from the hake shows some resemblances to *P. olssoni* in that the proportion of the body width to length is just slightly over 1 : 7, and the

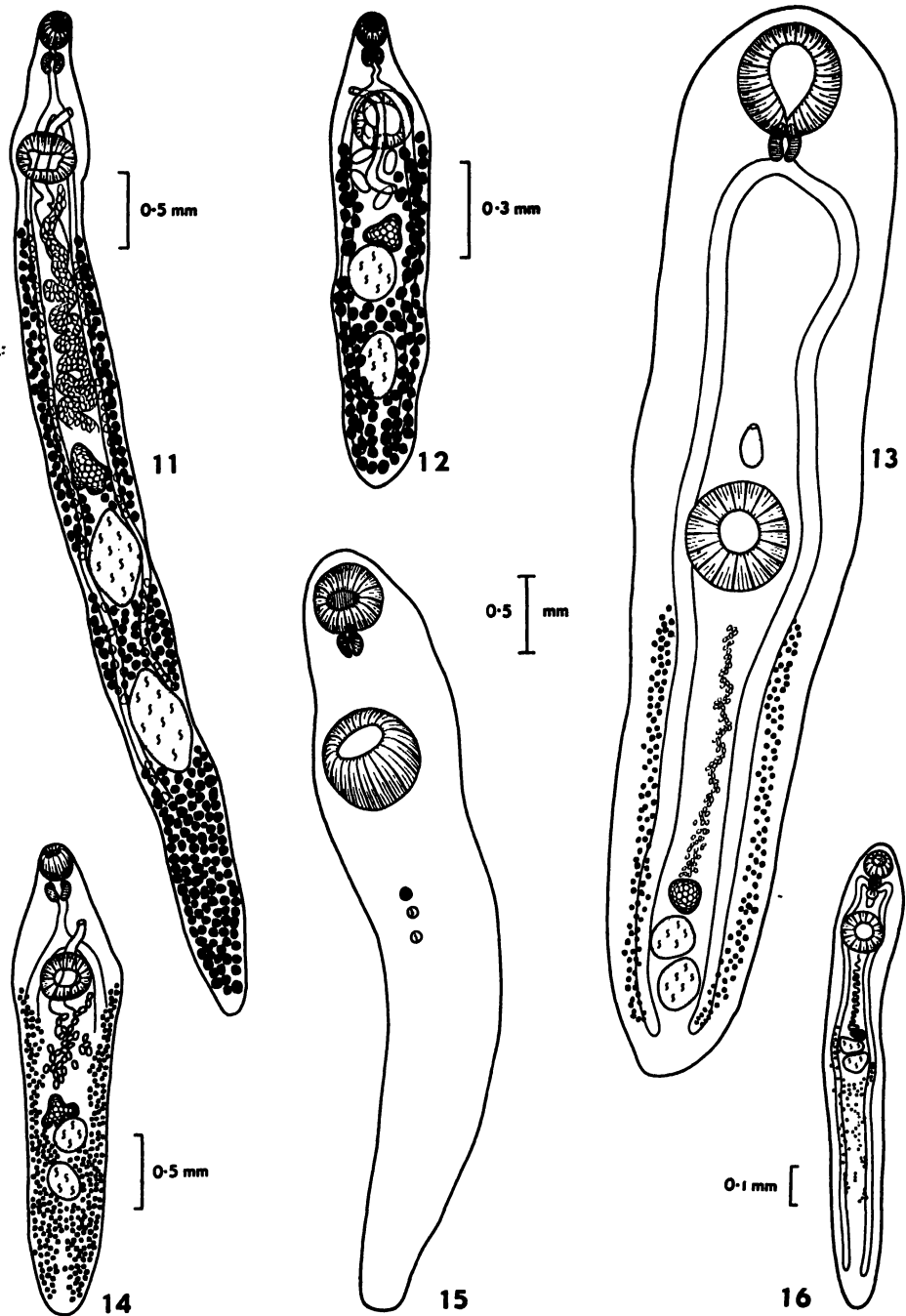


FIG. 11. *Podocotyle reflexa*, ventral view. FIG. 12. *Podocotyle staffordi*, dorsal view.
 FIG. 13. *Azygia angusticauda*, ventral view. FIG. 14. *Podocotyle aloman*, ventral view.
 FIG. 15. *Otodistomum cestoides* (*Xenodistomum melanocystis* of Stafford), immature.
 FIG. 16. *Otodistomum cestoides*, ventral view.

more anterior position of the ovary suggests that the cirrus sac, which cannot be seen, may reach half-way the distance from the acetabulum to the ovary. On the other hand the oesophagus in this specimen, as in *P. reflexa*, is definitely longer than the pharynx. As the specimen from the hake showed considerable contraction at the anterior end, the similarities to *P. olssoni* were very likely due to this. The difficulty encountered in attempting to assign this specimen to the proper species points out the desirability of using characters other than those of size alone in differentiating trematode species.

Members of this species are long, narrow, parallel-sided forms. The specimen from the hake was the smallest, measuring 3.6 mm. long. Mature specimens from the sea raven measured from 4.5 to 7.5 mm. in length. The oral sucker is terminal and about half the size of the subspherical acetabulum which is situated not far from the anterior tip of the body. There is a short pre-pharynx, a well developed pharynx, and an oesophagus which is usually longer than the pharynx. The oesophagus divides a short distance anterior to the acetabulum, and the narrow intestinal caeca extend to near the posterior tip of the body. The ovary is situated approximately halfway down the body length, or may be slightly anterior or posterior to this position. It usually shows three lobes. The uterus lies anterior to the ovary and leads to the genital pore situated on a level with the intestinal bifurcation, on the left side of the body. The vitellaria extend laterally a short distance behind the acetabulum to the posterior tip of the body, and, as is characteristic of this species, fill in the inter-testicular spaces, and are discontinued laterally in the region of the testes. The testes are large, spindle to oval shaped, situated in the posterior half of the body. The elongate cirrus pouch extends about a third of the distance from the acetabulum to the ovary. The measurements of one of the larger specimens is as follows: length 7.0 mm., width 0.59 mm., oral sucker 0.2 mm. in diameter, pharynx 0.17 mm. long, oesophagus 0.23 mm. long, acetabulum 0.33 by 0.41 mm., ovary 0.37 by 0.27 mm., anterior testis 0.68 by 0.39 mm., posterior testis 0.75 by 0.41 mm., eggs 0.084 by 0.042 mm.

Podocotyle staffordi sp. nov.

(Fig. 12)

(*Sinistroporus simplex* in part, of Stafford)

Host: *Gasterosteus aculeatus* (stickleback), from the intestine.

The collection contains a number of small specimens of a *Podocotyle* species from the stickleback. They are labelled *D. simplex*, but upon study showed characters that place them in a distinct species for which the name *P. staffordi* is proposed.

Podocotyle staffordi is most closely related to *P. olssoni*, which it resembles a great deal, but from which it differs by the character of its vitellaria and its smaller size. *P. staffordi* has the vitellaria filling the inter-testicular space but unlike *P. olssoni* the vitellaria are not discontinued laterally in the region

of the testes. Manter (10) refers to specimens of *P. olssoni* from the cod that appear to resemble *P. staffordi* in their size and in the fact that the lateral break in the vitellaria in the region of the testes does not always occur. The relationship of these forms to *P. staffordi* cannot be determined until the two forms are carefully compared. Nicoll (11) reported specimens of *P. atoman*, from the stickleback, which are very similar in size range to *P. staffordi*. However, he does not give the distribution of the vitellaria, and it seems likely that he was dealing with specimens of *P. staffordi*.

Specimens in the collection measure from 1.07 to 1.51 mm. in length, and are from five to six times as long as broad. The oral sucker is terminal, and spherical to subspherical in outline. The acetabulum is situated from one-fifth to one-quarter the distance from the anterior tip; it is spherical and about twice the diameter of the oral sucker. There is a short pre-pharynx, a well developed pharynx, and an oesophagus that is somewhat longer than the pharynx. The narrow intestinal caeca extend to near the posterior tip of the body. The three-lobed ovary is situated just anterior to the middle of the body. The uterus passes anterior from the ovary to the genital pore situated on a plane with the intestinal bifurcation, on the left side of the body. The testes are situated in the second half of the body in tandem arrangement; they are oval in outline and separated by several rows of vitellarian follicles. The vitellaria extend laterally from the posterior margin of the acetabulum to the posterior tip, filling the inter- and post-testicular spaces; they are not interrupted laterally in the region of the testes. The cirrus pouch extends about half the distance from the acetabulum to the ovary. The type specimen shows the following measurements: length 1.42 mm., width 0.28 mm., oral sucker 0.084 mm. in diameter, acetabulum 0.17 mm. in diameter, pharynx 0.052 mm. long, ovary 0.1 by 0.1 mm., anterior testis 0.16 by 0.13 mm., posterior testis 0.18 by 0.11 mm., eggs 0.091 by 0.045 mm.

Stenakron vetustum Stafford, 1904

(Fig. 10)

Host: *Hemitripterus americanus* (sea raven), from the intestine.

Stafford proposed the genus and species names *Stenakron vetustum* for a trematode from *Limanda ferruginea* which Linton (5) described but did not name. Stafford gives no description but refers to the description of Linton as follows:—

"Body smooth, fusiform, thickset about the middle tapering nearly equally to each end. Anterior sucker subterminal, circular, aperture somewhat triangular in preserved specimens, acetabulum, a little in front of the middle, larger than oral sucker, aperture nearly circular. Pharynx subglobular, close to oral sucker, oesophagus distinct. Intestinal rami simple, extending to ovary. Vitellaria distributed in the middle regions of the body from testes

to pharynx. Testes two, rather large, placed a little diagonally on the median line near posterior end of body. Ovary smaller than testes, subglobular or slightly lobed, situated in front of the anterior testis and to the right. Ova, few, large, in front of ovary. Cirrus pouch to the right of acetabulum. Genital aperture about half-way between acetabulum and oral sucker, to right of median line, at about midway between pharynx and acetabulum. Dimensions of living specimen, in millimetres; length 2.57; diameter, anterior 0.25, middle 0.93; posterior 0.19; diameter of oral sucker 0.21, of acetabulum 0.36; anterior testis, length 0.43, breadth 0.36; posterior testis, length 0.43, breadth 0.37; ova, 0.065 and 0.041 in the two principle diameters."

The collection contains two specimens, one of which is badly contracted. Both specimens are overstained and the details of the alimentary tract and the cirrus pouch cannot be made out. The organs that can be seen agree quite well with Linton's description. The one important exception is in the character of the ovary. In the one specimen in which the ovary can be seen clearly it consists of three separate and distinct oval parts. Thus there are apparently three ovaries. Whether this holds true for all specimens of this species cannot of course be stated until further specimens are studied. However, Linton's diagrams of this species could be interpreted as having two ovaries. Stafford's specimens differ further from the Linton's description in the larger size of the eggs and the testes. They show the following measurements: body sizes 1.60 by 0.70 mm. and 1.50 by 0.72 mm. respectively, diameters of oral suckers 0.16 and 0.19 mm., diameters of acetabula 0.24 and 0.30 mm., pharynx 0.09 mm., anterior testis 0.39 by 0.24 mm., posterior testis 0.41 by 0.26 mm., eggs 0.077 by 0.036 mm.

Stenakron vetustum has not been assigned to any definite group of trematodes and Poche (17) lists it under "unclassified general". In its general morphology it agrees mostly nearly with members of the family Alloecreadiidae where it probably belongs.

Genus *Plagioporus* Stafford, 1904

Synonyms: *Lebouria* Nicoll, 1909

Caudotestis Yamaguti, 1934

Plagioporus serotinus Stafford, 1904

Host: *Moxostomata* sp. (red horse sucker).

Stafford gave the name *Plagioporus serotinus* to a trematode he obtained from the intestine of the red horse sucker. As the author has pointed out (9) this species is valid as is the genus, and *Lebouria* Nicoll, 1909, and *Caudotestis* Yamaguti, 1934, are synonyms of *Plagioporus*.

The material in the collection consists of three specimens none of which bear eggs. They range from 1.34 to 1.65 mm. in length and are approximately one-fourth as broad as long. The species has been fully described elsewhere (9) and will not be discussed further here.

Stenocollum fragile (Linton, 1900)

(Fig. 3)

Host: *Mola mola* (sunfish), from the intestine.

The genus *Stenocollum* was created by Stafford to include *Distomum fragile* (Linton, 1900). Stafford gave no description but referred to the description of Linton which reads as follows:—

"Body unarmed, fusiform from acetabulum back, depressed, neck elongated, slender, cylindrical, slightly enlarged at mouth. Acetabulum a little larger than mouth, subglobular, at base of neck sessile; mouth terminal, or nearly so; pharynx subglobular, situated a distance equal to twice its length or more behind the posterior edge of the oral sucker, followed by a slender oesophagus; intestinal crura simple, beginning in the neck about half-way between the pharynx and acetabulum, extending to near the posterior end of the body; testes two, median, approximate, situated near the posterior end of the body, a little longer than broad; ovary subtriangular in outline, lying immediately in front of the anterior testis and a little to the right; cirrus and cirrus pouch immediately in front of the acetabulum and to the left; vitellaria very abundant, appearing in subangular masses at posterior end and along dorsal and lateral regions of the body to and even in front of the acetabulum; uterine folds between acetabulum and ovary; ova relatively large and in moderate number."

"Dimensions of mounted specimen in millimetres: length 1.78, diameter of anterior sucker 0.10, diameter of neck behind mouth 0.07, diameter of acetabulum 0.24, greatest diameter 0.33, distance of acetabulum from anterior end 0.71, diameter of acetabulum 0.14, length of testis 0.17, breadth 0.14, diameter of ovary 0.10, longer diameter of ovum 0.069, shorter diameter 0.038, length of pharynx 0.06, distance between pharynx and anterior sucker 0.15."

The collection contains several specimens of this species, which are, unfortunately, badly faded. Only the body shape, the distribution of the vitellaria and the uterus, and the size of the eggs, can be made out with certainty. For this reason very little can be added to Linton's description. However, the specimens in the collection measure, on the average, slightly more than 3 mm. in length with the proportion of the thin neck region to the thicker main part of the body being as 1.5–2 : 1. The width of the body at the widest part ranges from 0.33 to 0.41 mm. The eggs measure 0.0612 to 0.0693 mm. in length by 0.0386 to 0.0408 mm. in width.

Poche (17) included the genus *Stenocollum* with his genera *Fasciolidorum sedis incertae*. From the characters presented by Linton (4) it can readily be included in the family Allocreadiidae. It shows many resemblances to the genus *Anallocreadium* Simer, 1929, and upon further study it may be possible to demonstrate that *S. fragile* does not have a true cirrus pouch, as is the case in *Anallocreadium*.

FAMILY HEMIURIIDAE

Hemiurus levinseni Ohdner, 1905

(Fig. 19)

(*Hemiurus appendiculatus* in part, of Stafford)Hosts: *Gadus callarias* (cod)*Clupea harengus* (herring), from the oesophagus and stomach.

The collection contains 10 specimens of this species labelled *D. ocreatum*, but which, from the host list and the fact that he makes no mention of *D. ocreatum*, Stafford must have included in his species *H. appendiculatus*. The specimens from the cod are larger than those from the herring, an average specimen measuring 2.36 mm. in length. An average specimen from the herring measures 1.45 mm. in length. The specimens can be recognized as *H. levinseni* by the fact that the two suckers are approximately equal, while they are obviously unequal in the species *H. appendiculatus* and *H. communis*.

Brachyphallus crenatus Rudolphi, 1802

(Fig. 18)

(*Hemiurus appendiculatus* in part, of Stafford)Hosts: *Salmo salar* (salmon)*Hippoglossus hippoglossus* (halibut)*Osmerus mordax* (smelt)*Reinhardtius hippoglossoides* (greenland turbot), from oesophagus, stomach, and intestine.

The 12 specimens in the collection were all labelled *D. appendiculatus* and were apparently placed by Stafford together with *H. levinseni* in the species *H. appendiculatus*. The average specimens measure from 1.5 to 2.0 mm. in length in an extended condition. However, the single specimen from the Greenland turbot measures 3.2 mm. in length. The width is about one-fifth the length. While the specimens vary in the structure of the vitellaria, some being lobed and others smooth-margined, there appears to be only one species and not two as stated by Looss (7).

Derogenes varicus Muller, 1788

(Fig. 17)

Hosts: *Salmo salar* (salmon)*Gadus callarius* (cod)*Osmerus mordax* (smelt)*Anguilla chrysypa* (eel)*Cottus scorpius* (sculpin)*Hippoglossus hippoglossus* (halibut)*Reinhardtius hippoglossoides* (Greenland turbot)*Melanogrammus aeglefinus* (haddock), from the oesophagus and stomach.

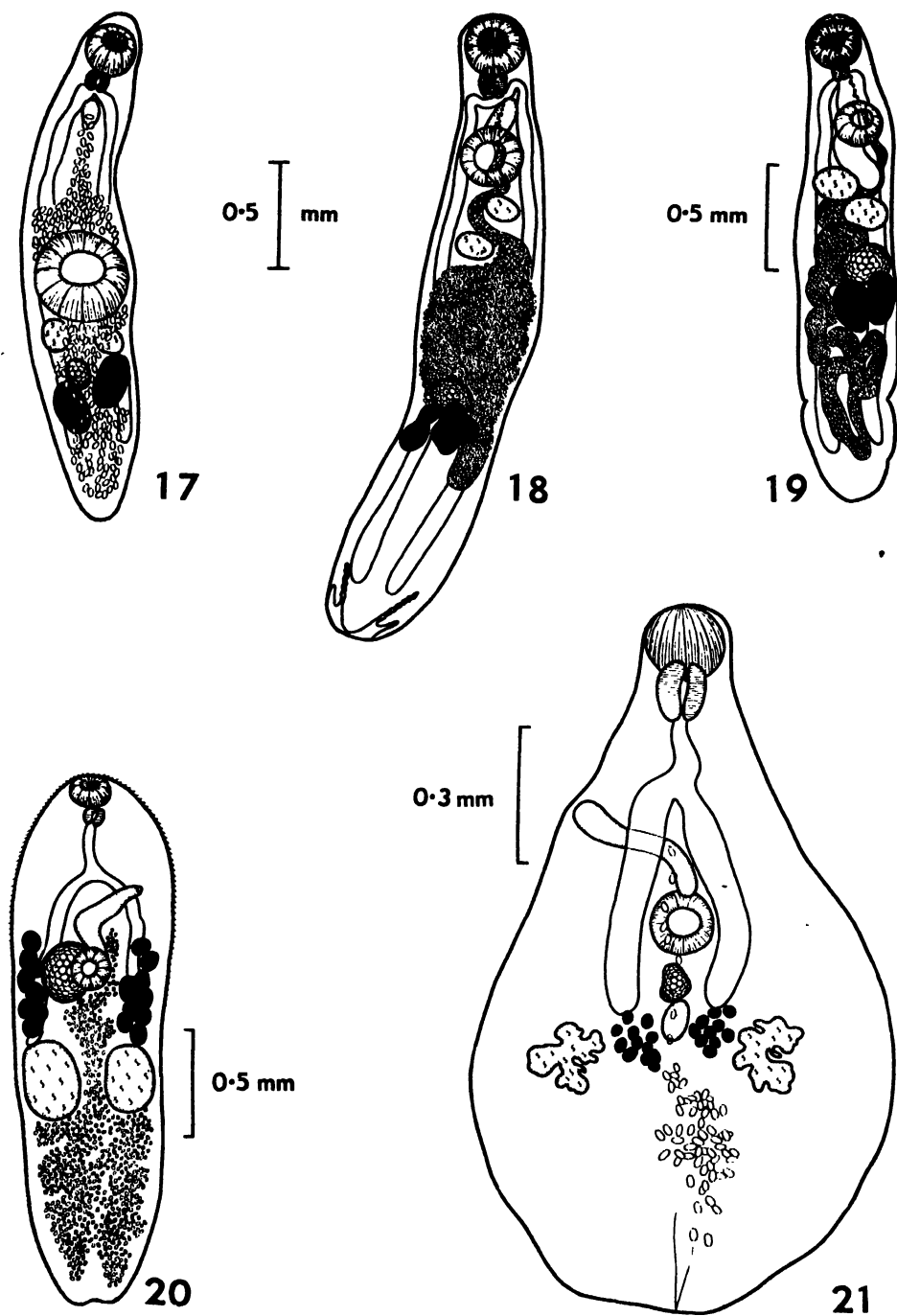


FIG. 17. *Derogenes varicus*, ventral view. FIG. 18. *Brachyphallus crenatus*, ventral view.
 FIG. 19. *Hemiurus levinsoni*, ventral view. FIG. 20. *Steganoderma formosum*, ventral view.
 FIG. 21. *Lepidophyllum steenstrupi*, dorsal view.

This species is the most numerous in the collection. Specimens range from 1.3 to 3.0 mm. in length, and in morphology agree essentially with the excellent description of Ohdner (13).

Specimens of the species *Derogenes plenus*, created by Stafford, could not be found in the collection.

FAMILY FELLODISTOMIDAE

Genus *Fellodistomum* Stafford, 1904

Fellodistomum fellis Olsson, 1868

(*Fellodistomum incisum*, of Stafford)

(Fig. 7)

Host: *Annahrichas lupus* (wolf fish).

Although Nicoll (11) accepts Stafford's name for the genus *Fellodistomum*, he points out that *F. fellis* is a different species from *Distomum incisum* Rudolphi, 1809. Moreover because of Stafford's description stating that the ovary is on the left side of the body, and the testes large and spherical in outline, Nicoll doubts whether he was dealing with *F. fellis*. The specimen in Stafford's collection, however, shows the ovary on the right side of the body, and the testes obliquely oval; thus there is little doubt that it was *F. fellis*.

The collection contains a single immature specimen, which measures 3.48 by 1.8 mm. It is broadly ovate in outline. The oral sucker is subterminal, and measures 0.45 mm. in diameter. The acetabulum is situated slightly posterior to the middle of the body; its diameter is more than twice that of the oral sucker, it is spherical in outline, and measures 0.98 mm. in diameter. The pharynx is 0.16 mm. long. There is no pre-pharynx or oesophagus. The broad intestinal caeca extend to near the posterior margins of the testes. The testes are situated midway between the acetabulum and the posterior extremity. They are side by side with the left testis in a slightly more posterior position. They are obliquely oval in outline and are separated by their own width. The ovary is situated immediately anterior to the right testis on the right side of the body. The genital opening lies slightly to the left of the mid-line, midway between the anterior margin of the acetabulum and the intestinal bifurcation. The broad cirrus pouch lies almost wholly in front of the acetabulum. The vitellaria form a narrow band on either side of the acetabulum, extending slightly anterior and posterior to this organ.

Fellodistomum agnotum Nicoll, 1909

(*Fellodistomum incisum* of Stafford?)

(Fig. 8)

Host: *Annahrichas lupus* (wolf fish), from the gall bladder.

The collection contains two mature and one immature specimen. Whether Stafford includes these in his *F. incisum* cannot be stated definitely as the

slides are not labelled. However, as he makes no special mention of them it seems most likely that he included them in *F. incisum*.

Felodistomum agnotum was described as a new species by Nicoll (11), who differentiated it from *F. fellis* by the smaller size of the ventral as compared to the oral sucker, the more dorsal position of the vitellaria, and the more elongate body. The specimens in the collection are roughly spindle-shaped forms somewhat attenuated anteriorly. The oral sucker is spherical and subterminal. The acetabulum is larger than the oral sucker but its diameter is less than twice that of the latter, it is usually flattened in the antero-posterior axis. There is a well developed pharynx which leads directly into the broad intestinal caeca. The intestinal caeca extend to the posterior margin of the right testis. The two somewhat lobed testes are situated in the third quarter of the body in oblique arrangement, the left testis being more anteriorly placed. The lobed ovary is situated immediately anterior to the right testis. The uterus passes back to the posterior tip of the body, then passes forward to the genital pore situated on the right of the mid-line, midway between the acetabulum and the intestinal bifurcation. The cirrus pouch overlaps the acetabulum for a short distance. The vitellaria extend laterally from the intestinal bifurcation to the posterior margin of the acetabulum. Measurements of the largest mature specimen are as follows: length 2.9 mm., width 1.09 mm., oral sucker 0.29 mm. in diameter, pharynx 0.14 mm. long, acetabulum 0.42 mm. by 0.46 mm., eggs 0.044 by 0.024 mm.

The material in the collection differs from Nicoll's description in the more posterior extent of the vitellaria and the oblique arrangement of the testes, characters that are probably only variations within a species.

Stringophora furciger Olsson, 1868

(Fig. 9)

(*Leioderma furcigerum* of Stafford)

Hosts: *Reinhardtius hippoglossoides* (Greenland turbot)

Pseudopleuronectes americanus (winter flounder)

Cryptacanthodes maculatus (wry mouth).

Stafford (19) created the genus *Leioderma* for *Distoma furciger* Olsson. However, *Leioderma* is preoccupied by *Leioderma* Willemoes-Suhm, 1873, thus *Stringophora* Ohdner, 1905, becomes the proper genus.

The collection contains 10 specimens. Mature specimens vary from 1.24 to 4.0 mm. in length and are from one-fifth to one-third as wide as long. The oral sucker is subspherical, measuring 0.35 to 0.41 mm. in diameter, and is situated one-third of the body length from the anterior tip of the ventral sucker; it is broader than long and measures 0.4 by 0.6 mm. The pharynx is 0.18 mm. long. The oesophagus is slightly longer than the pharynx. The intestinal caeca extend a short distance posterior to the testes. The testes are oval in outline and are arranged side by side at approximately the middle of the body. The ovary is a highly lobed structure, situated slightly

to the right of the mid-line between the testes and the acetabulum, and usually overlapping the latter organ to a slight extent. The uterus extends back to the posterior tip of the body. The genital pore lies on the mid-line, a short distance anterior to the acetabulum. The cirrus pouch is broadly oval in outline. It overlaps the acetabulum to about one-fifth of its length. The vitellaria are limited to a space between the posterior third of the acetabulum and the posterior margins of the testes. The eggs measure 0.055 by 0.029 mm.

FAMILY ZOOGONIDAE

Steganoderma formosum Stafford, 1904

(Fig. 20)

Host: *Hippoglossus hippoglossus* (halibut), from the intestine.

This species was described by Stafford who created for it the new genus *Steganoderma*. Manter (10) points out the close resemblance of *Steganoderma* to *Lecithostaphylus* Ohdner, 1911, but differentiates them on the basis of the difference in shape of the cirrus pouch, the difference in size of the pharynx, the oesophagus, and the excretory bladder, and the presence or absence of a Laurer's canal pore. It is doubtful whether these characters are sufficiently constant or important to justify the retention of the genus *Lecithostaphylus*.

The collection contains a single specimen, which measures 2.69 mm. by 0.76 mm. It is rounded at the anterior tip and somewhat truncate at the posterior extremity. The cuticula is covered with small closely set spines that are more prominent in the anterior part of the body. The oral sucker is flattened antero-posteriorly and measures 0.2 by 0.15 mm. The pharynx is small, measuring 0.063 mm. in length. The length of the oesophagus is more than twice that of the pharynx, and it divides into the intestinal caeca about half-way between the suckers. The intestinal caeca extend to the anterior margins of the testes. The acetabulum is situated about two-fifths of the body length from the anterior tip; it is subspherical and measures 0.2 mm. in diameter. The testes are arranged side by side just below the middle of the body length, and are broadly ovate in shape. The right testis is somewhat the larger, measuring 0.37 by 0.29 mm., whereas the left testis measures 0.35 by 0.25 mm. The ovary is ovate in shape; it is situated to the right of the mid-line, almost on a level with the acetabulum and partially overlapping that organ. The uterus almost fills the entire post-testicular region. The genital pore is situated on the left side of the body on a level with the intestinal bifurcation. The vitellarian follicles are large and few in number; they extend laterally from slightly anterior of the acetabulum to the anterior margin of the testes. The narrow cirrus pouch overlaps the acetabulum a very short distance. The eggs measure 0.036 by 0.017 mm.

FAMILY AZYGIIDAE

Genus *Azygia* Looss, 1899Synonyms: *Megadistomum* Stafford, 1904*Mimodistomum* Stafford, 1904

Stafford created two new genera and one new species for the members of the genus *Azygia* he found in freshwater fish. Ohdner (16) correctly points out that there are insufficient grounds for the creation of the two new genera, and considers *Megadistomum* and *Mimodistomum* synonymous with *Azygia*.

Azygia longa (Leidy, 1851)(*Mimodistomum longum* and *Azygia tereticolle*, of Stafford)Hosts: *Esox masquinongy* (muskallunge)*Lota maculosa* (ling), from the stomach.

The collection contains two specimens of this species, one from the muskallunge and one from the ling. The specimen from the muskallunge is undoubtedly Stafford's *Megadistomum longum*. It is a large well preserved specimen measuring 18.5 by 1.2 mm. It does not contain any eggs and is undoubtedly referred to by Stafford when he states with regard to this species: "Alcoholic specimens measuring 18 mm. in length and over 1.0 mm. wide, have genital glands developed but no eggs."

The specimen from the ling, though not labelled, is undoubtedly *A. tereticolle* of Stafford. The specimen measures 6.5 by 0.5 mm. and contains eggs. Manter (10) points out that *A. tereticolle* is synonymous with *A. longa*.

Azygia angusticauda (Stafford, 1904)

(Fig. 13)

(*Mimodistomum angusticaudum*, of Stafford)Host: *Lota maculosa* (ling), from the stomach.

The collection contains two mature and several immature specimens. The two mature specimens measure 7.25 by 1.65 mm. and 7.0 by 1.2 mm., respectively. The oral sucker is terminal, spherical to subspherical in outline. There is a well developed pharynx that opens directly into the intestinal caeca. The intestinal caeca extend to the posterior tip of the body. The acetabulum is about the same size as the oral sucker, or may be slightly smaller, and is situated about half-way down the body length. The genitalia are crowded towards the posterior tip of the body, the testes being posterior to the ovary and in tandem to oblique arrangement. The ovary is immediately anterior to the anterior testis. The uterus lies between the ovary and the genital pore, which is situated on the mid-line a short distance anterior to the acetabulum. The vitellaria extend laterally from a short distance posterior to the acetabulum to the posterior end of the body. One of the specimens shows the following measurements: length 7.25 mm., width 1.65 mm., oral sucker 0.83 by 0.72 mm., acetabulum 0.72 mm. diameter, pharynx 0.29 mm. long, anterior testis 0.35 mm. diameter, posterior testis 0.39 by 0.33 mm.

Genus *Otodistomum* Stafford, 1904*Otodistomum cestoides* van Beneden, 1871

(Fig. 16)

(*Otodistomum veliporum* and *Xenodistomum melanocystis*, of Stafford)Host: *Raia stabuliforis* (barn door skate), from the stomach and intestine.

Stafford founded the genus *Otodistomum* to include what he thought was *D. veliporum* Creplin, 1837. Ohdner (16) and Manter (10) believe Stafford's species to be *O. cestoides*. The more constant differences between these two species appears to be the egg size and the size of the genital papilla. Unfortunately Stafford's specimens are all immature. However, in one specimen a prominent genital papilla, characteristic of *O. cestoides*, can be seen. Furthermore, from their distribution and host it seems most likely that Stafford's specimens are *O. cestoides*.

The collection contains three immature specimens, the largest of which measures 12.5 by 1.6 mm. The measurements given below refer to this specimen. The body is parallel-sided with rounded ends. The oral sucker is subterminal, flattened in the antero-posterior axis, and measures 0.72 by 0.79 mm. The pharynx is well developed, measuring 0.31 mm. in length. The caeca originate immediately behind the pharynx and extend to the posterior end of the body. The acetabulum is situated about one-fifth the length of the body from the anterior tip; it is larger than the oral sucker, measuring 1.14 by 1.24 mm. The genitalia are situated slightly anterior to the middle of the body, the testes are in approximate tandem arrangement, and the ovary lies immediately anterior to the anterior testis and slightly to the left of the mid-line. The uterus lies between the ovary and the genital pore, which is situated just posterior to the pharynx. The vitellaria consist of small scattered follicles concentrated for the most part posterior to the genitalia. The genital opening has a conspicuous protruding papilla.

Stafford created the genus and species *Xenodistomum melanocystis* for immature trematodes he found encysted in the stomach wall of *Lophius piscatorius* (goose fish). From the general appearance of the immature specimens in the collection from the goose fish, they can be referred to the genus *Otodistomum*, and are probably immature specimens of *O. cestoides*. This is the conclusion reached by Ohdner (16).

FAMILY GORGODERIDAE

Lepidophylum steenstrupi Ohdner, 1902

(Fig. 21)

Host: *Annahrichas lupus* (wolf fish), from the urinary bladder.

The collection contains five specimens, only one of which harbours eggs. The body of this form is flattened, sac-shaped, with a broadly rounded posterior end from which it tapers to the anterior end. It is widest at the level of the testes. The cuticula is covered with fine spines. The oral

sucker is terminal and subspherical in outline. There is a large muscular pharynx, an oesophagus of about the same length, and two intestinal caeca that extend to slightly more than half-way down the length of the body. The acetabulum is slightly smaller than the oral sucker and is situated just anterior to the middle of the body. The ovary is somewhat lobed, and is situated just posterior to the acetabulum. The testes are deeply lobed, situated one on either side of the mid-line, with their anterior margins just below the level of the ovary. The vitellaria consist of a few follicles arranged in two groups, between, and slightly anterior to, the testes. The uterus passes back to the posterior tip of the body. The genital pore is situated near the right margin of the body, on a level a little below the intestinal bifurcation. The elongate cirrus pouch reaches back to the anterior margin of the testes. The mature specimen shows the following measurements: length 1.5 mm., width 0.9 mm., oral sucker 0.17 mm. in diameter, pharynx 0.126 mm. long, acetabulum 0.15 mm. in diameter, eggs 0.04 by 0.02 mm.

Phyllodistomum folium van Olfers, 1816

Host: *Esox lucius* (pike), from the urinary bladder.

The collection contains a single specimen with the posterior part of the body completely filled with eggs, which obscure the internal anatomy. The specimen measures 1.21 by 0.74 mm. The oral sucker is 0.130 mm. in diameter, the ventral sucker being somewhat larger measures 0.163 mm. in diameter. The eggs measure 0.032 by 0.016 mm.

As Stafford points out, his specimen differs from the description of *P. folium* in the size of the two suckers. Furthermore, the size of the eggs is smaller in his specimen. However, it does resemble *P. folium* in the general size and shape of the body and was recovered from the same host. Until further specimens from the same host can be studied, it is thought best to consider it as *P. folium*.

FAMILY HETEROPHYIDAE

Cryptogonimus diaphanus (Stafford, 1904)

(Fig. 24)

(*Protenteron diaphanum*, of Stafford)

Host: *Ambloplites rupestris* (rock bass), from the intestine.

Stafford described a trematode from the intestine of the rock bass, as a new species, and referred it to the new genus *Protenteron*. His collection does not contain any specimens that exactly fit his description. However, there is one specimen from the rock bass that was collected in October, 1904, and therefore after his paper was published, which is very probably the same species. Stafford's description is as follows:

"A living specimen measured 1.54×0.385 . Broadest at the middle, narrowing behind. Mouth-sucker terminal, 0.186. Ventral sucker small 0.062 (Stafford's figure of 0.62 is obviously a misprint), situated 0.57 from the anterior end. Skin with fine spines. Pre-pharynx longer than either

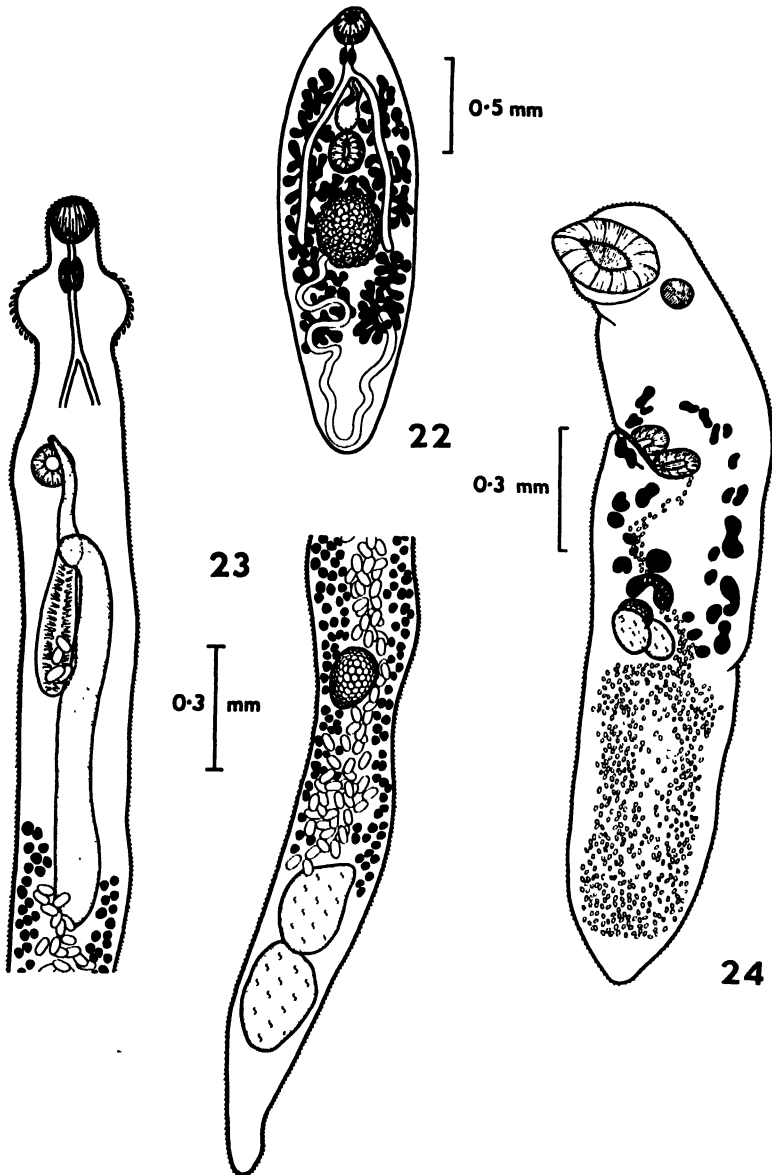


FIG. 22. *Centrovarium lobotes*, ventral view. FIG. 23. *Deropristis inflata*, dorsal view.
FIG. 24. *Cryptogonimus diaphanus*.

pharynx or oesophagus. Short caeca diverging and falling short of ventral sucker or scarcely passing it. Black eyespots lateral from pharynx. Testes obliquely side by side in middle of post-acetabular body. Ovary a little in front of left testis. Uterus reaching to posterior end—older eggs in the folds to the left. Egg 0.022 by 0.011 (again Stafford's figure of 0.22 for the egg length is obviously a misprint). Genital sucker close in front of ventral

sucker. Penis apparatus reaching behind to the ovary. Vitellaria lateral, short, from the forking of the intestine to near the ovary."

The specimen in the collection is poorly stained, and as the body is twisted it is difficult to make out all the organs. It measures 1.9 by 0.4 mm. The cuticula is covered with fine spines. Eyespots are present. While the pre-pharynx cannot be made out, the pharynx is some distance from the oral sucker, suggesting that the pre-pharynx is fairly long. The oral sucker is about three times the size of the acetabulum. The testes are obliquely side by side in the middle of the post-acetabular body, and the ovary appears to be in front of the left testis although this is difficult to make out as the specimen is twisted laterally. The vitellaria extend from the intestinal bifurcation to the level of the ovary. The uterus reaches to the posterior tip of the body. The egg size is the same as that given by Stafford, 0.02 by 0.01 mm. The penis apparatus mentioned by Stafford cannot be seen, nor are the intestinal caeca visible. However, the genital pore is surrounded by a muscular sucker, a character Stafford failed to observe.

Cryptogonimus diaphanus is undoubtedly closely related to *C. chyli*, which occurs in the same host. It resembles it in the spiny cuticula, the presence of eyespots, the distribution of the genital organs, and in the extent of the intestinal caeca. Furthermore, the muscular genital atrium-like structure is very much like that found in *C. chyli*. It differs from *C. chyli* in the larger size, the smaller genital organs, and non-lobate ovary, and in the presence of an elongate pre-pharynx.

The specimen of *Cryptogonimus* at hand shows the following measurements: length 1.9 mm., width 0.4 mm., oral sucker 0.22 mm. by 0.14 mm., testes 0.09 and 0.10 mm. in length, the right and left, respectively, ovary somewhat smaller than the testes, eggs 0.2 by 0.1 mm. The exact measurements of the acetabulum could not be obtained. However, it appeared to be slightly less than one-third the size of the oral sucker. The pharynx is about the same size as the acetabulum.

Centrovarium lobotes (McCallum, 1895)

(Fig. 22)

Host: *Esox lucius* (pike), *Stizostedion vitreum* (doré), from the stomach.

Stafford created the genus *Centrovarium* to include *Distomum lobotes* McCallum. More recently Van Cleave and Mueller (21) made a careful study of this form and placed it in the family Heterophyiidae.

The collection contains four specimens, three from the pike and one from the doré. The body of these parasites is oval with rounded ends; the anterior body region is usually the broader. They range from 1.6 to 2.3 mm. in length. The terminal oral sucker is spherical in outline. There is a short pre-pharynx, a small pharynx, and an oesophagus about the same length as

the pharynx. The intestinal caeca extend about half-way down the body length. The acetabulum is situated slightly less than a third of the body length from the anterior tip. The large rosette-shaped ovary is situated in the middle of the body. The testes are arranged one on each side of the body, a short distance posterior to the ovary. The uterus passes back to the posterior tip of the body. The genital pore lies on the mid-line, anterior to the acetabulum. The vitellaria extend from the intestinal bifurcation to within a short distance posterior to the ovary. The follicles are greatly branched and usually meet dorsally at the mid-line. The prominent cirrus sac may extend posterior to the acetabulum; the eggs measure 0.03 by 0.13 mm.

FAMILY ACANTHOCOLPIDAE

Deropristis inflata Molin, 1859

(Fig. 23)

Host: *Anguilla chrysa* (eel), from the intestine.

Stafford reported two species of *Deropristis* from Canadian fishes. However, the collection contains only three specimens of *D. inflata*. Ward (22, pp. 509-521) doubts Stafford's record of *D. hispidus* from the sturgeon. The specimens in the collection show his record of *D. inflata* to be accurate.

The specimens at hand are rather on the large side, ranging from 3.4 to 3.7 mm. in length. They are long narrow forms with an expanded collar bearing a row of large spines near the anterior tip. The cuticula is covered with smaller spines throughout. The small oral sucker is spherical. There is an obvious pre-pharynx and a medium-sized pharynx which is somewhat shorter than the diameter of the acetabulum. The oesophagus is longer than the pharynx and forks into the narrow intestinal caeca a considerable distance anterior to the acetabulum. The intestinal caeca extend to within a short distance of the posterior tip. The acetabulum is situated about a fifth of the distance from the anterior tip. It is spherical, and about the same size as the oral sucker. The oval testes are in tandem arrangement near the posterior tip. The oval ovary lies about half-way between the posterior tip of the cirrus sac and the anterior testis. The uterus passes back to the anterior margin of the anterior testis. The genital pore is situated immediately anterior to the acetabulum, on the mid-line. The cirrus pouch extends back to the middle of the body. The vitellaria extend laterally from the posterior margin of the cirrus pouch to the anterior testis. The measurements of the best preserved specimen are as follows: length 3.4 mm., width 0.26 mm., oral sucker 0.10 mm. in diameter, pharynx 0.08 mm. long, acetabulum 0.10 mm. in diameter, ovary 0.2 by 0.09 mm., anterior testis 0.22 by 0.15 mm., posterior testis 0.26 by 0.16 mm., cirrus pouch 0.97 mm. long, eggs 0.045 by 0.024 mm.

Stephanostomum sp.

HOSTS: *Hemitripterus americanus* (sea raven)
Cryptocanthodes maculatus (wrymouth)
Lycodes sp., from the intestine.

The specimens are all somewhat faded and it is impossible to count the circum-oral spines accurately. The specimens from the sea raven range from 2.2 to 2.5 mm. in length, the specimen from the wrymouth measures 1.8 mm., and that from *Lycodes* 2.7 mm. in length. The specimens from the sea raven appear to have from 45 to 50 spines arranged in two rows, the spines of the anterior row being smaller. In general morphology these specimens approach *Stephanostomum baccatus* Nicoll, 1907, and together with the character of the circum-oral spines they show a great resemblance to that species. The specimen from *Lycodes* appears to have comparatively small spines around the oral opening, although in general appearance it also resembles *S. baccatus*.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

FEBRUARY, 1941

NUMBER 2

EFFECT OF TEMPERATURE AND HUMIDITY ON COLOUR OF LEAN, AND DEVELOPMENT OF RANCIDITY IN THE FAT, OF PORK DURING FROZEN STORAGE¹

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Abstract

Pork cuts were stored at relative humidities of 83, 87, 92, 96, and 100% and temperatures of -6.6° , -12.2° , -17.7° , and -23° C. for 48 weeks. Visual examination revealed various degrees of surface drying under all conditions, and pronounced methaemoglobin formation at -6.6° C. Quantitative colour measurements on both the exposed and internal surfaces of the lean, showed that storage temperature was the primary factor affecting the colour. Temperatures of -18° C., or lower, are required to prevent these changes. Samples that had suffered the greatest colour change during storage showed the least change during subsequent exposure.

Temperature was also the primary factor affecting the development of rancidity in the fat. Both peroxide oxygen and free fatty acid increased significantly with increase in storage temperature, particularly between -12.2° and -6.6° C., but the actual quantities of free fatty acid were small and of little consequence. Storage temperatures of -18° C., or lower, are essential if spoilage of pork fat is to be avoided over storage periods of approximately one year's duration.

Introduction

In Canada considerable quantities of pork must be stored in the frozen condition for the subsequent manufacture of Wiltshire bacon during periods when hog receipts are limited. Since this material must be thawed, cured, and exported after storage, rather exacting storage conditions are required. Microbial activity, methaemoglobin formation, and the development of rancidity in the fats are the principal forms of deterioration to be expected at the higher freezer temperatures. The low relative humidity prevailing in most freezers also causes surface drying, the condition responsible for freezer burn and loss of bloom. This investigation was undertaken to obtain quantitative information on the change of bloom and pigment in the lean, and spoilage of the fat, of pork stored under various temperature and humidity conditions.

¹ Manuscript received November 5, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. A portion of the material was presented by one of the writers (W.H.W.) in a thesis to the University of Western Ontario in partial fulfilment for the degree of Master of Arts. Issued as paper No. 57 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 966.

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Methods

The method employed for maintaining the required temperature and humidity conditions was that used in similar investigations on poultry (1). Small cuts of prime pork back were placed in desiccators containing calcium chloride solutions of such concentrations as to maintain the desired relative humidities. The containers were stored in refrigerated rooms at the desired temperatures. Every precaution was taken to insure that the effective relative humidity in each container corresponded as closely as possible to that computed from the vapour pressure of the calcium chloride solution. All surfaces of the pork that could not be seen during inspection were covered with waxed paper to prevent evaporation. Since small samples were used, the area of evaporating surface was negligible compared with the exposed area of the solution; thus it was insured that the humidity corresponded to that of the solution, and excessive dilution of the solution during storage was avoided. To avoid condensation on the inner surfaces, all samples were frozen before they were placed in the containers.

Duplicate samples were stored at 83, 87, 92, 96, and 100% relative humidity at each of the storage temperatures -6.6° , -12.2° , -17.7° , and -23° C. (20° , 10° , 0° , and -10° F.). The values of the relative humidity represent the means of the initial and final values as computed from the corresponding density measurements.

The colour measurements were made with the colour comparator previously described (4). A single measurement was made on each exposed surface and duplicate measurements on internal surfaces. An estimate of colour stability was obtained from measurements on the internal surfaces after exposure for 17 and 89 hr. at 10° C. and 95 to 100% relative humidity.

Peroxide oxygen and free fatty acid determinations were made on samples of the fat prepared by procedures to be described in detail in another paper. In brief, chopped samples of the fat, after drying by admixture with anhydrous sodium sulphate, were extracted in a Soxhlet apparatus for two hours with redistilled petroleum ether. Most of the ether was subsequently removed by distillation, and final traces by heating *in vacuo* at 40° C. The peroxide oxygen and free fatty acid, taken as estimates of oxidative and hydrolytic changes respectively, were determined as previously described (2).

Results of Visible Examinations

Periodic examinations were made during the storage period for surface drying, discoloration, and mould development. A loss of bloom was evident in all samples after 8 to 12 weeks' storage. In the samples stored at low humidities and high temperatures, this condition gradually developed into freezer burn of various degrees of severity. Quantitative estimates of the extent of freezer burn were therefore impossible. Methaemoglobin formation and mould development occurred on all samples stored at -6.6° C. The final examination confirmed these observations and yielded slight evidence of methaemoglobin formation in certain samples stored at -12.2° C.

Colour Measurements

It was felt that colour measurements on the exposed and internal surfaces after storage might permit quantitative expression of the observed changes in bloom and pigment. Although all three colour components, i.e., red, green, and blue, were measured for all samples, it is unnecessary to report the detailed results. It has already been shown that the scatters of the individual components are correlated (6), and although a significant element of independent fluctuation can be demonstrated when a large number of measurements are available, the data obtained in these experiments were considered inadequate for this purpose. This correlation between the components was demonstrated, in these experiments, by the fact that if one component was affected significantly by one of the storage conditions, the other components generally showed the same behaviour. In these circumstances, only the results on the total scatter for all components (brightness) are presented except in a few instances where a single component showed significant effects not revealed by the total scatter. Subsequent tables show the results obtained at each temperature, averaged for all humidities, and those for each relative humidity, averaged for all temperatures. This is supplemented with an analysis of variance for each component separately. A similar treatment was used for all fat measurements.

TABLE I

SURFACE COLOUR OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean brightness, for all relative humidities at each storage temperature					
Temperature, ° C.	- 6.6	-12.2	-17.7	-23.0	
Brightness (total, %)	58.5	53.0	46.2	46.6	
Mean brightness, for all temperatures at each relative humidity					
Relative humidity, %	83	87	92	96	100
Brightness (total, %)	49.1	49.4	51.9	55.0	49.8
Analysis of variance					
Variance attributable to	Degrees freedom	Mean square			
		Blue	Green	Red	Brightness (total)
Between temperatures	3	12.9 **	79.5 **	37.7 **	324 **
Between humidities	4	3.11	5.01	8.79	47.8
Residual ¹ (T × H)	12	1.79	6.16	6.51	37.8

**Indicates 1% level of significance.

¹ Residual (T × H) did not significantly exceed error of duplicates.

The results reported in Table I show that the samples gradually darkened in colour as the temperature decreased from -6.6° to -17.7° C. There was also some indication that the brightness decreased with relative humidity. The analysis of variance shows that the difference due to storage temperature was the only one that attained statistical significance; all components and their totals showed significant effects.

Table II shows the results of similar measurements on the internal surfaces. Storage temperature was again the primary factor affecting colour, although significant effects could not be demonstrated for the red component. The green component alone showed significant differences following storage at the various humidities, the values being somewhat lower at the two lowest relative humidities. Since the samples were rather small, the effect of surface drying and discoloration may have penetrated in some samples to the depth at which these internal colours were measured. Had it been possible to obtain samples at a considerable depth, significant effects of relative humidity, at least, would hardly be expected.

The colour measurements on the internal samples are of interest for comparing the observed changes in the exposed samples. The measurements on the internal surfaces of the samples stored at -17.7° and -23° C. showed that

TABLE II

INTERNAL COLOUR OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean brightness and green scatter, for all relative humidities at each storage temperature					
Temperature, $^{\circ}$ C.	- 6.6	-12.2	-17.7	-23.0	
Brightness (total, %)	57.8	51.4	54.1	54.0	
Green scatter, %	17.5	15.4	16.2	16.0	
Mean brightness and green scatter, for all temperatures at each relative humidity					
Relative humidity, %	83	87	92	96	100
Brightness (total, %)	53.1	52.2	55.7	55.8	54.7
Green scatter, %	15.8	15.6	16.5	17.0	16.6
Analysis of variance					
Variance attributable to	Degrees freedom	Mean square			
		Blue	Green	Red	Total
Between temperature	3	6.09*	16.5 **	12.6	137 **
Between humidities	4	2.78	7.10*	3.11	40.7
Residual ¹ (T \times H)	12	1.22	2.20	4.90	38.7

*Indicates 5% level of significance.

**Indicates 1% level of significance.

¹ Residual (T \times H) did not significantly exceed error of duplicates.

the colour was satisfactory, and can be considered as unchanged from the original. The brightness of these samples was about 54%. By comparison, the brightness of exposed surfaces must have increased during storage at -6.6°C ., remained constant when stored at -12.2°C ., and darkened at the lower storage temperatures. The increase in brightness at -6.6°C . is attributed to methaemoglobin formation, a fact confirmed by observations made on other samples. The darkening observed following storage at -17.7° and -23°C . must be caused by the drying or loss of bloom, the only change observed at these temperatures. Earlier studies (3) have shown that drying at temperatures above the freezing point is accompanied by darkening, and a similar behaviour apparently applies to drying from the frozen state. The value of 53% obtained for the brightness of exposed surfaces of samples stored at -12.2°C . does not differ appreciably from 54% and suggests that the surface of the samples remained unaltered. Since drying did occur on the surface of all samples, darkening would be expected. Apparently sufficient brightening was obtained by methaemoglobin formation to offset the darkening caused by drying. The change in the brightness of the internal samples was less marked, and is doubtless attributable to similar effects.

Colour Stability Measurements

The internal surfaces were exposed, under conditions described previously, for periods of 17 and 89 hr. and again measured. The change from the initial value over each of these periods was taken as an estimate of colour stability. The general tendency was for the samples to become brighter during the 17-hr. period, and to become even darker than the initial value following the 89-hr. period. In expressing and analyzing the results, an increase in brightness is reported as a positive, and darkening as a negative, value.

The initial average brightness, and that following each exposure period, together with an analysis of variance on the change in colour scatter for each component for each period, appears in Table III. Storage temperature was the only factor showing a significant effect on colour stability. There is also evidence of differential effects or changes in colour quality, since the change in green scatter and total brightness after 17-hr. exposure was not related to the previous storage temperature, while the change in the red component was not significant after 89-hr. exposure. For the samples stored at all temperatures, an initial increase in brightness followed by a decrease is evident, but the initial brightness and the extent of the changes vary markedly.

A complete interpretation of these data is impossible at the present time. Presumably the formation of methaemoglobin during storage at the higher temperatures was responsible for the increased brightness. During exposure for a short period at a higher temperature, this process continues, but is least marked in samples that have already suffered a partial change in this direction. The final darkening may be attributable to drying, although this may not be so in view of the high humidity maintained in the exposure chamber. In any event, the extent of the darkening appears to be conditioned

TABLE III

COLOUR STABILITY OF INTERNAL SURFACE OF PORK AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Storage temperature, °C.	Exposure period	Observed colour (total scatter for all components)	Colour stability (change in total scatter from initial value)
- 6.6	0	56.7	—
	17	57.9	1.2
	89	55.3	-1.4
-12.2	0	51.5	—
	17	56.2	4.7
	89	49.7	-1.8
-17.7	0	54.2	—
	17	57.3	3.1
	89	49.7	-4.5
-29.0	0	54.0	—
	17	56.4	2.4
	89	47.3	-6.7

Analysis of variance
(After 17-hr. exposure)

Variance attributable to	Degrees freedom	Blue	Green	Red	Total
Between temperatures	3	4.44*	3.31	6.38**	19.1
Between humidities	4	0.54	3.05	0.89	10.4
Residual ¹ (T × H)	12	0.66	1.27	0.76	6.68

After 89-hr. exposure

Between temperatures	3	18.2 *	23.9 **	3.93	121 **
Between humidities	4	0.76	2.97	4.93	21.7
Residual ¹ (T × H)	12	4.78	2.08	2.15	12.1

*Indicates 5% level of significance.

**Indicates 1% level of significance.

¹ Residual (T × H) significantly exceeds error of duplicates in few instances.

by the extent of brightening due to methaemoglobin formation during previous storage or exposure. Previous results (5, 7) indicate that initially bright samples of bacon are most likely to suffer darkening during exposure. The opposite effect observed in the present study may be attributed to a difference between pork and bacon, which seldom becomes brighter, or because the increased brightness was due to methaemoglobin as distinct from a lower pigment level.

TABLE IV

PEROXIDE OXYGEN CONTENT OF PORK FAT AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean content of peroxide oxygen for all relative humidities at each storage temperature

Temperature, ° C. Peroxide oxygen*†	- 6.6 19.9	-12.2 1.45	-17.7 0.54	-23.0 0.46
----------------------------------------	---------------	---------------	---------------	---------------

Mean content of peroxide oxygen for all temperatures at each relative humidity

Relative humidity, % Peroxide oxygen*†	83 6.99	87 5.47	92 5.05	96 4.57	100 5.83
-------------------------------------------	------------	------------	------------	------------	-------------

Analysis of variance

Variance attributable to	Degrees freedom	Mean square
Temperature	3	455 ***
Humidity	4	3.38
Residual ¹ (T × H)	12	3.36

†As ml. of 0.002N sodium thiosulphate.

*Necessary difference, 5% level of significance: 1.97.

***Indicates 0.1% level of significance.

¹ Residual (T × H) did not significantly exceed error of duplicates.

TABLE V

FREE FATTY ACID CONTENT OF PORK FAT AFTER STORAGE FOR 48 WEEKS AT VARIOUS TEMPERATURES AND RELATIVE HUMIDITIES

Mean content of free fatty acid for all relative humidities at each storage temperature

Temperature, ° C. Free fatty acid†*	-6.6 1.15	-12.2 0.67	-17.7 0.48	-23.0 0.37
----------------------------------------	--------------	---------------	---------------	---------------

Mean content of free fatty acid for all temperatures at each relative humidity

Relative humidity, % Free fatty acid†*	83 0.67	87 0.66	92 0.67	96 0.67	100 0.65
-------------------------------------------	------------	------------	------------	------------	-------------

Analysis of variance

Variance attributable to	Degrees freedom	Mean square
Temperature	3	1.19 ***
Humidity	4	0.001
Residual ¹ (T × H)	12	0.001

†As % oleic acid.

*Necessary difference, 5% level of significance: 0.03.

***Indicates 0.1% level of significance.

¹ Residual (T × H) did not significantly exceed error of duplicates.

Development of Rancidity

Temperature was the primary storage condition affecting the formation of peroxide oxygen in the fat (Table IV). Although an increase in temperature was in all instances associated with an increase in the peroxide oxygen content, the observed differences were statistically significant only for a change from -12.2° to -6.6° C. The mean values for each temperature at all relative humidities show that for periods comparable to that studied here, pork should be stored at -12.2° C. (preferably at -17.7° or -23° C.) if rancidity in the fat is to be avoided. There was some indication that peroxide oxygen formation decreased with increase in relative humidity, but the differences noted were not statistically significant. It is considered that temperature and not the presence of moulds, was the prime factor responsible for the high peroxide oxygen content observed at -6.6° C.

Storage temperature alone had a significant effect on free fatty acid formation (Table V). It may be seen, from the mean values at each temperature over all relative humidities, that successive decreases in temperature were in each instance associated with statistically significant decreases in the free fatty acid content. It is to be noted, however, that even at temperatures as high as -6.6° C., the content of free fatty acid was small, and would contribute little to spoilage in pork fat. Variations in the relative humidity had no apparent effect on free fatty acid formation.

Acknowledgments

The authors wish to acknowledge the assistance of Messrs. A. E. Chadderton, E. A. Rooke, and E. G. Blake, Laboratory Assistants, and to express their thanks to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice on statistical matters.

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CANADIAN WILTSHIRE BACON

XV. QUANTITATIVE BACTERIOLOGICAL AND CHEMICAL CHANGES IN TANK PICKLE AND ON BACON DURING CURE AND MATURATION¹

BY N. E. GIBBONS² AND W. HAROLD WHITE³

Abstract

Little or no change due to time or depth was found in the bacterial content of Wiltshire tank pickle during cure. A decided increase in the number of bacteria occurred on the surface of the sides. Settling of bacteria from the pickle was not responsible for this increase. Of the normal commercial practices of salting the sides prior to cure, and washing and wiping after cure, wiping was the most important in reducing the bacterial population of the surface of the meat.

The concentration of sodium chloride, nitrate, and nitrite in the curing pickle decreased most rapidly during the first 12 or 24 hr. of cure. Over the remainder of the curing period, the chloride content decreased at a relatively uniform rate and the nitrite content remained constant within the sampling error. Changes in nitrate were generally less than the sampling error. Although stratification of the salts in the tank was evident even after three hours from the beginning of cure, the actual magnitude of the differences was small and would have little detrimental effect on the bacon. There was no apparent relation between bacterial and chemical changes in the pickle during cure.

Introduction

It is generally believed that the presence of bacteria is essential for the conversion of pork to bacon. If so, the conditions prevailing in the curing of Wiltshire sides, namely, the high salt concentration in the curing pickles, the low temperature of the curing cellars (3.3° to 4.5° C.), and the short cure (six to eight days), are favourable for the rapid growth of only halophilic and psychrophilic organisms. From previous studies (4) it appeared that the bacterial count of curing pickles increased slightly during cure, but the data did not indicate whether multiplication was just beginning or whether a rapid development and decline had occurred. The present investigation was undertaken to determine some of the bacteriological and chemical changes taking place during the curing of Wiltshire bacon, and, in addition, to study the effect of certain plant practices, such as salting, washing, and wiping, on bacterial development.

Methods

All the experiments were performed under actual plant conditions. The composition and treatment of the pickles, pumping practice, and cure were those normally employed in the plant. The sides selected for sampling formed

¹ Manuscript received October 12, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 56 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 965.

The results given in this paper were reported in part at the 3rd International Congress of Microbiology, New York, Sept. 5, 1939 (Proceedings, p. 703, 1940).

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part of the regular cure. Although the curing tanks were only four to five feet deep, it was considered that differences in the bacterial and chemical composition of the cover pickle might exist between the top and bottom of the tank. Provision was therefore made for securing samples of the curing pickle at these two levels.

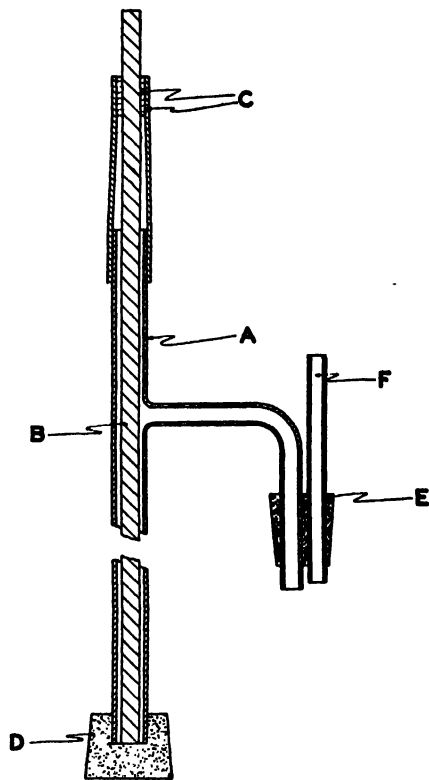


FIG. 1. *Device employed for obtaining samples of curing pickle from curing vats.*

To insure that the samples were drawn from the required depth without contamination, the sampling device shown in Fig. 1 was employed. It consisted of a glass tube, *A*, of suitable length, bearing a side-arm approximately three inches from the top and containing a stout glass rod, *B*, approximately six inches longer than *A*. Rubber tubing, *C*, between the glass tube and rod served to maintain an air tight connection at the top while permitting the displacement of the cork, *D*. In use the cork was pushed off by means of the rod, a sterile flask placed in position at *E* and the pickle sample removed from the desired depth by applying suction at *F*.

In order to obtain samples near the bottom of the tank, tubes, corresponding to the number of samples required, were fastened to a wooden frame, the side-arms were wrapped in paper, and the whole assembly was sterilized in a large sterilizer. The corks, *D*, were then covered with sterile paraffin wax, followed by a layer of Parafilm. Waxed paper was also placed around the wrapped

side-arms. The wooden frame, which was fastened in the curing tank, allowed sides to be packed closely around it and at the same time insured that all samples were taken from the same depth. Top samples were removed by lowering single unmounted tubes to the desired depth.

The media used and the general methods employed for the bacteriological study of pickles and bacon have been described (4, 5). Plates were incubated at 20° C., unless otherwise stated. For bacteriological examination of the meat, 12 sq. cm. of the pleural membrane, comprising 3 sq. cm. from each of the third, sixth, ninth, and twelfth ribs, were taken at each sampling. Physiological saline (the salt concentration to which the organisms would normally be subjected) was used to dilute meat samples taken from sides before cure. However, a 4% salt solution was used for the studies on cured meat since it has been shown that at this concentration there was the greatest development of the organisms found on bacon (5).

In one instance an attempt was made to assess the number of bacteria present in curing pickle which were capable of growing under anaerobic conditions. Using the same dilutions and media as for aerobic counts, plates were poured and incubated at room temperature (24° C.) under anaerobic conditions obtained by the method of Weiss and Spaulding (6). Nutrient agar plates were counted after 12 to 14 days, and 4 and 10% salt agar plates after 18 to 21 days.

The methods employed for the determination of the concentration of chloride nitrate, and nitrite in bacon and curing pickle have been given (7).

Experimental Procedure

Two samples of the pump pickle were taken approximately one hour apart from the pump needle. The cover pickle was sampled as it entered the tank, first when the tank was about half-filled and again when nearly filled. During cure the cover pickle was sampled for chemical analysis 2, 5, 9, and 14 hr. after the tank had been filled and daily thereafter. Samples for bacteriological analysis were taken two hours after filling and then after one, three, five, and six days. Two sets of samples were obtained on each occasion from sampling tubes located about two feet from each wall and in diagonally opposite corners of the 14 by 15 ft. curing tank. Samples were obtained from two depths at each position, namely, 12 and 42 in. below the surface, the latter being about 10 inches from the bottom. After curing for six days, the cover pickle was drained from the tank, the sides were allowed to drain overnight, and then refloated with the mixed Wiltshire pickle from the storage tank. This was the only departure from the regular routine, as the sides are not usually allowed to drain in the tank.

Four sides were sampled for surface bacterial content about four hours before placing in the tank. These, together with two unpumped sides, were placed midway up the stack of sides near one of the positions from which the pickle samples were taken. They were not resampled until after removal

from the tank. After draining for one day in the draining cellar at $4.4^{\circ}\text{C}.$, the sides were baled without wiping and stored at $1.1^{\circ}\text{C}.$ for 16 days, i.e., 24 days from the beginning of cure. The ribs and gammons were sampled after removal from cure and after storage for bacteriological and chemical analysis.

Changes During Cure

Curing Pickle

The results of the chemical analysis appear in graphical form in Fig. 2. The two curves appearing for each constituent represent the decrease in composition at the two depths, averaged for both horizontal positions in the tank. The cross hatched section represents the decrease in concentration necessary to exceed the 5% level of statistical significance, as computed from the error of sampling the tank, i.e., between positions. The magnitude of the differences in concentration of the salts between the top and bottom of this shallow tank was quite small, and the stratification would have little detrimental effect on the quality of the bacon.

The most rapid changes in concentration of all three curing salts occurred during the first 24 hr. of cure. Over the rest of the curing period the chloride

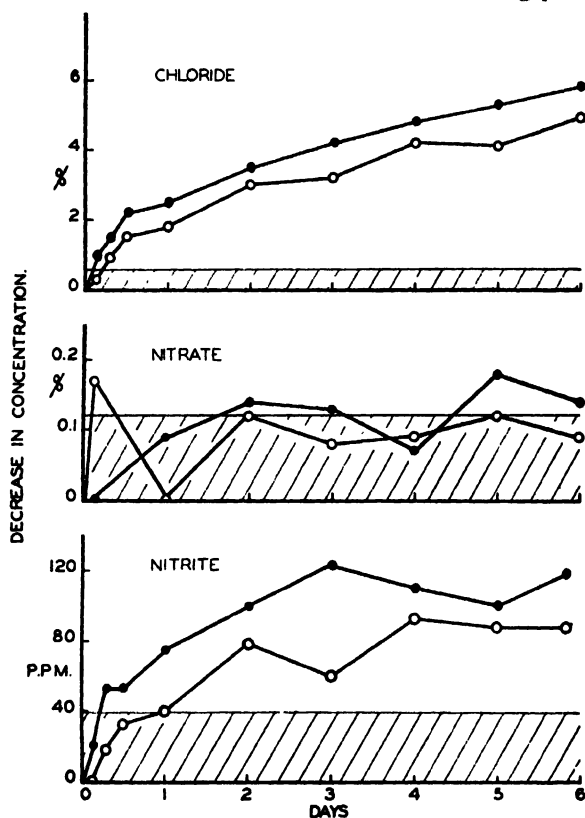


FIG. 2. Decrease in the concentration of sodium chloride, nitrate, and nitrite in Wiltshire tank pickle during cure. ● indicates values for top position in the curing tank, ○ for bottom position. The cross hatched section represents the necessary difference from zero decrease.

content decreased at a relatively uniform rate, whereas the concentration of nitrite remained practically constant. It is reasonable to assume that the diffusion of nitrite into the tissue continued throughout cure. This, together with the destruction of nitrite by nitrite reducing bacteria, appears to balance approximately its formation by nitrate reducing bacteria. Although the nitrate content decreased slightly, the observed changes were usually not significantly greater than the sampling error. For the most part, the changes in the concentration of the three salts at the two depths studied were comparable.

TABLE I

ANALYSIS OF VARIANCE FOR CHANGES IN SODIUM CHLORIDE, NITRATE, AND NITRITE CONTENT OF WILTSHIRE TANK CURING PICKLE DURING CURE

Variance attributable to	Chloride		Nitrate		Nitrite	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Time	9	26.8**	7	0.0189*	9	11,800**
Depth	1	10.0**	1	.0023	1	13,700**
Sampling error	20	0.15**	16	.0066**	20	700
Residual	49	.03	39	.0002	49	500

* Indicates 5% level of significance.

** Indicates 1% level of significance.

Statistical analyses were made to determine the significance of the differences attributable to the various factors studied. The variance could be analysed into portions attributable to the effect of time, depth, position, the differential effects of these factors, and the analytical error derived from duplicate tests in the laboratory. Although the differential effects were usually significantly greater than the residual variance, they did not differ significantly within themselves or from the variance between horizontal positions. Consequently the estimate of sampling error used for statistical comparison here was obtained by grouping the variance due to horizontal position in the tank with the first order interactions between the various factors studied. The results of the analysis showed that, for chloride and nitrite, the effect of both time and depth were significantly greater than the sampling error; for nitrate (Table I), time alone had a significant effect.

Bacteriological analysis showed little difference between two samples of pump pickle taken from the pump needle one hour apart. Average logarithmic counts of 5.84 and 5.26, were obtained on 10 and 4% salt agar and of 4.58 and 3.90 on nutrient agar at 20° and 37° C., respectively.

The samples of cover pickle taken as it was flowing into the tank were practically identical in bacterial content with those removed from the tank. The average logarithmic count was 6.95 and 6.33, on 10 and 4% salt agar and 5.17 and 4.13 on nutrient agar at 20° and 37° C., respectively.

The results of the bacteriological analyses during cure are presented in Fig. 3 and Table II in the same form as the chemical data. From the graph (Fig. 3, Tank 1), in which the logarithm of the average number of organisms found in the two positions is plotted against time for each of the four media, it is evident that the changes are small, and in some instances, somewhat irregular.

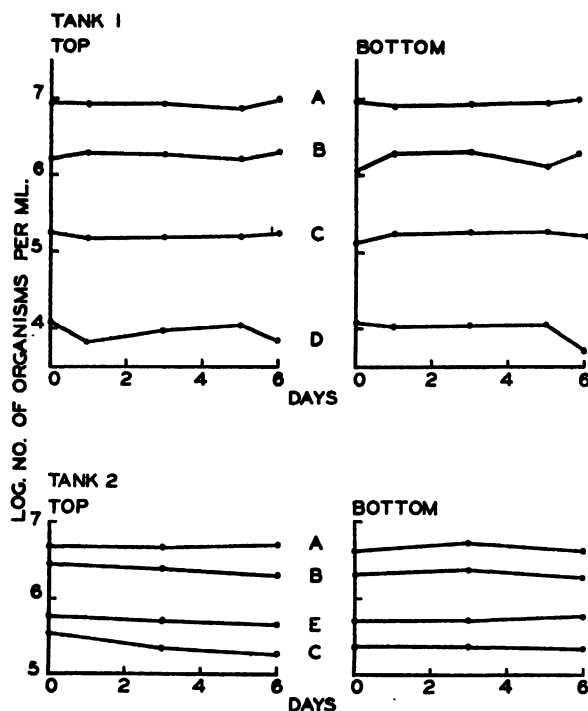


FIG. 3. Changes during cure in bacterial content of Wiltshire pickle in two tanks (1 and 2) as estimated on (A) 10% salt agar at 20° C., (B) 4% salt agar at 20° C., (C) nutrient agar at 20° C., (D) nutrient agar at 37° C., and (E) 20% salt agar at 20° C.

Statistical analysis of the logarithms of the number of organisms found on four combinations of media and incubation temperatures indicate that the effect of time alone was significant. Moreover, this was so only for those organisms capable of growth on nutrient agar at 37° C. and on 4% salt agar at 20° C. Since the significant changes were irregular, an interpretation of their nature is impossible.

The bacteriological changes during cure in a later experiment (page 70) are also shown in Fig. 3 (Tank 2). In this instance a medium containing 20% salt was used but only about one-tenth as many organisms developed as on 10% salt agar. On no medium was an increase in the number of organisms found during cure.

The results presented here failed to confirm a previous observation that spent pickle contained three to four times as many organisms as cover pickle (4). In the previous investigation the majority of the pickle samples were

TABLE II

ANALYSIS OF VARIANCE FOR CHANGES IN BACTERIAL CONTENT OF WILTSHIRE TANK PICKLE DURING CURE

Variance attributable to	D.f.	Mean square			
		Dist. water nut. agar 37° C.	Dist. water nut. agar 20° C.	4% brine 4% salt agar 20° C.	10% brine 10% salt agar 20° C.
Time	4	0.193**	0.00919	0.0739*	0.0163
Depth	1	.028	.00226	.0029	.0287
Sampling error	13	.031**	.00472**	.0145**	.0064**
Residual	40	.004	.00045	.0011†	.0005

* Indicates 5% level of significance.

** Indicates 1% level of significance.

† 38 degrees of freedom.

obtained by dipping from the surface. It was therefore considered possible that differences might exist in the bacterial content of surface and subsurface layers of the curing pickle. To test this supposition, samples were taken at the surface by dipping with a flask and from just below the surface by the sampling device described earlier. In addition, samples were taken prior to the entry of the pickle into the tank and upon the removal of the sides.

The results (Table III) show that most of the surface samples contained slightly more organisms than those from the subsurface. However, the difference would account for only a small proportion of that found previously. Since the bacterial content of the pickle was greater after the sides had been removed than before the tank was disturbed, it is possible that the washing of organisms from the sides may account in part for increases observed in the population of the pickle. However, since this pickle was pumped off and

TABLE III

EFFECT OF SAMPLING POSITION ON THE NUMBER OF ORGANISMS IN WILTSHIRE TANK PICKLE. LOGARITHM OF THE NUMBER OF ORGANISMS PER MILLILITRE

Sample	Nut. agar		10% salt agar	
	Days		Days	
	0	8	0	8
1	5.21 ¹	5.66 ²	6.24 ¹	6.44 ²
2A	5.17	4.96	6.12	6.01
2B	5.27	5.09	6.20	6.09
3A	5.19	4.90	6.21	6.06
3B	5.25	5.00	6.21	6.10

¹ Taken from brine line.² Taken from tank after sides removed; mixed with other pickle.

A. Samples from 6 in. below the surface.

B. Samples dipped up from surface in flask.

mixed with other pickle prior to floating the sides, the increase might be due to the added pickle. It is of interest to note that in this particular cure the bacterial content of the spent pickle was in all instances lower than that of the cover pickle.

The results obtained for the number of organisms in curing pickle which were capable of anaerobic growth (Table IV) indicated a slight decrease in number during the early part of cure, followed by an increase. The depth in the tank at which the sample was taken had little apparent effect. Although no attempt has been made to classify the types of organisms present, the majority were capable of aerobic growth and were therefore facultative anaerobes.

TABLE IV

THE NUMBER OF ORGANISMS CAPABLE OF ANAEROBIC GROWTH IN PUMP PICKLE AND IN TANK PICKLE DURING CURE, EXPRESSED AS LOGARITHM OF THE NUMBER OF BACTERIA PER MILLILITRE

Medium	Top			Bottom			Pump pickle	
	Days						A	B
	0	3	6	0	3	6		
Position 1								
Nut. agar 24° C.	3.62	3.31	3.99	3.49	3.28	3.90	3.27	3.11
4% salt agar	5.34	5.11	5.91	5.37	4.04	5.71	4.68	4.36
10% salt agar	5.77	5.31	6.18	5.87	4.40	6.02	4.75	4.50
Position 2								
Nut. agar 24° C.	—	3.00	3.95	3.82	3.16	3.81		
4% salt agar	5.58	4.88	4.90	5.48	4.90	5.83		
10% salt agar	6.10	5.58	6.04	6.06	5.10	6.06		

In the anaerobic chambers the methylene blue indicator usually decolorized overnight in the desiccators containing nutrient agar plates but, in jars containing salt agar plates, the decolorization was extremely slow and at times proceeded only to a green or pale green shade. Furthermore, the rate of decolorization decreased with increased salt concentration of the medium and with the proportion of salt agar plates in the desiccator. No improvement was obtained by evacuating again and filling the desiccators with hydrogen. Since the indicator was decolorized in jars containing no salt agar and slowly in those containing salt agar, it was assumed that anaerobic conditions prevailed in all. It has been found since that this difficulty may be avoided by decolorizing the indicator by heating before placing in the jars. The indicator then remains colourless until the jar is opened.

Bacon

During cure, a decided increase in the number of organisms on the sides was noted. The increase of those organisms capable of growth on 4 and 10% salt agar (Fig. 4) was especially high—100 to 200 times as compared with a maximum increase of 1.7 times in the tank curing pickle. During the storage of the sides after cure, the greatest increase was on 4% salt agar, the salt

concentration most closely approaching that of the bacon. However, in no instance was the rate of increase as great as during cure. This is due, in part, to the lower temperature of storage.

It was considered that the rapid increase in bacterial count on the rib surface of the sides during cure might be due to the settling of bacteria from the pickle. To test this possibility three sides (1 to 3) were placed in cure with the rib surface facing up as customary, and three (4 to 6) with the rib surface down. The results obtained (Table V) for bacterial counts on the rib surface before and after cure (eight days) showed there was a similar increase in the number of organisms regardless of the position of the rib

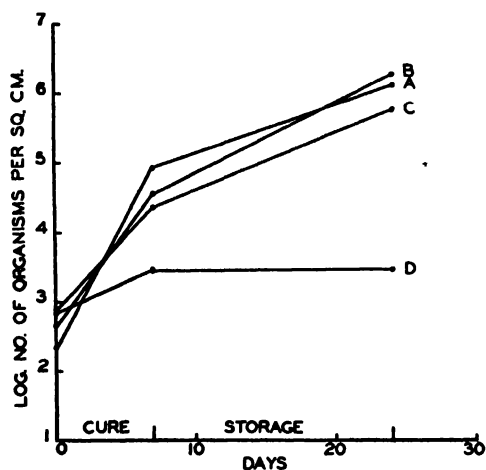


FIG. 4. Changes in the number of bacteria on the surface of Wiltshire bacon sides during cure (0 to 7 days) and storage (7 to 24 days) as estimated on (A) 10% salt agar, (B) 4% salt agar and (C) nutrient agar at 20° C., and on (D) nutrient agar at 37° C. Averages of four sides.

TABLE V

EFFECT OF POSITION OF RIB SURFACE OF WILTSHIRE BACON SIDES IN THE TANK DURING CURE ON THE NUMBER OF ORGANISMS PER SQUARE CENTIMETRE EXPRESSED AS THE LOGARITHM

Side	Before cure			After cure		
	Nut. agar	4% salt agar	10% salt agar	Nut. agar	4% salt agar	10% salt agar
1	3.34	3.48	2.90	4.32	4.58	4.64
2	3.62	3.54	2.90	4.71	5.18	5.33
3	4.45	4.22	3.66	4.56	5.79	5.97
4	3.63	3.43	3.00	4.11	4.37	4.56
5	2.79	2.63	2.43	5.91	—	5.83
6	2.86	2.79	2.64	4.45	4.64	4.83
1 Ham	4.43	4.41	4.25	5.15	5.25	5.32
2 Ham	4.43	4.43	4.23	6.15	6.26	5.55
3 Ham	4.85	4.77	4.87	5.76	6.19	5.53

Sides 1 to 3 cured with rib surface up, 4 to 6 with rib surface down.

surface in the curing tank. Samples removed from the exposed lean surface of the gammons both at the beginning and end of cure were usually higher in bacterial content than the ribs. During the cure the rate of increase was essentially the same on both rib and gammon surfaces. It is therefore concluded that factors other than the settling of bacteria from the pickle are responsible for the rapid increase during cure.

The data obtained for the concentration of the curing salts and the distribution of sodium chloride in the hams of pumped and unpumped sides have been given previously (8).

Effect of Washing, Wiping, and Salting on the Surface Bacterial Content of Bacon

In view of the increase in the number of bacteria on the sides during cure, the effect of three common plant practices, namely, salting the sides in the tank, washing, and wiping them after cure, was investigated. For this purpose twelve sides, sampled as previously described for the bacterial content on the rib surface, were divided into three groups of four each. When placed in one corner of the curing tank one group was sprinkled with the usual quantity of salt (16 oz. per side), another with approximately twice this amount, and the third with none. The heavily salted sides were placed nearest the bottom of the tank. Two hours after the beginning of cure and again after three and six days, pickle samples were removed from two positions just below the surface and from one approximately 10 in. from the bottom of the tank.

At the end of six days the sides were removed from cure, sampled, and divided into four groups of three each in such a manner that each group contained a representative of the three salt treatments. After the rib surfaces of two such groups were washed with fresh pickle from a hose, all the sides were drained overnight. On the following day one group of washed and one of unwashed sides were baled without further treatment, whereas the other two groups were, prior to baling, wiped with the customary white burlap cloths which had been washed in hot water at 93° C. (200° F.) and

TABLE VI

EFFECT OF DIFFERENT AMOUNTS OF SALT ON SIDES DURING CURE ON THE INCREASE IN THE NUMBER OF BACTERIA ON THE SURFACE. EXPRESSED AS LOGARITHM OF THE NUMBER OF ORGANISMS PER SQUARE CENTIMETRE, AVERAGE OF FOUR SIDES

	Before cure			After cure		
	Nut. agar	4% salt agar	10% salt agar	Nut. agar	4% salt agar	10% salt agar
Heavily salted	3.72	3.86	3.45	4.56	4.76	4.62
Regular salt	2.64	2.73	2.54	4.69	5.05	4.67
No salt	3.05	3.04	2.87	4.90	5.35	5.16

rinsed in pickle at 71° C. (160° F.) before use. In this manner a similar number of washed, washed and wiped, wiped, and untreated sides were obtained for comparison. After baling, the sides were stored at 1.1° C. for 12 days, a period comparable to shipment to England. After each of the above treatments, samples for bacteriological analysis were removed from the rib surface of each side.

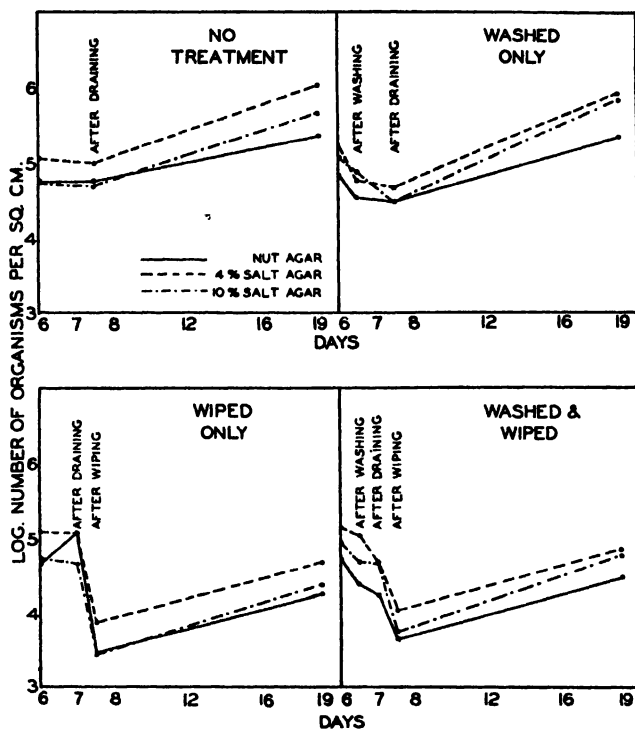


FIG. 5. Effect of washing, wiping, draining, and storage on the number of bacteria on the surface of Wiltshire sides. Time in days from the beginning of cure. Averages of three sides.

During cure the number of organisms on the sides increased in all instances (Table VI). The rate was greatest for the sides upon which no salt had been sprinkled and decreased with increasing amounts of salt. Although a random allocation of sides was made for the three groups, all those receiving the heavy salting had significantly higher initial counts than the remainder. Thus, although the final counts for these sides were approximately the same as the others, there is some indication from the growth rates that heavy salting inhibits surface bacterial growth on sides during cure.

The logarithms of the number of organisms per square centimetre of rib surface after the various treatments are shown graphically in Fig. 5. It is apparent that draining had little effect on the bacterial population. Washing caused a slight reduction in the number of organisms. Wiping, however, reduced the count to one-quarter to one-tenth of the original number.

The significance of the differences noted for these various operations was tested statistically. Since the number of bacteria on the sides at the end of cure did not differ significantly, direct comparisons of subsequent procedures can be made. A *t* test on the differences in the logarithm of the number of organisms before and after draining gave insignificant values (*t* being 0.33, 0.76, and 0.65 for nutrient agar, 4, and 10% salt agar, respectively).

TABLE VII

ANALYSIS OF VARIANCE OF DIFFERENCES IN LOGARITHM OF THE SURFACE COUNTS OF 12 SIDES OF WILTSHIRE BACON BEFORE AND AFTER TREATMENT

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Salting	2	1.51	2.25	2.06
Washing, etc.	3	9.91*	7.12	9.54*
Error	6	1.41	1.72	1.03

* Indicates 5% level of significance.

An analysis of variance of the differences in the logarithm of the counts before and after the different operations showed that the amount of salt sprinkled on the sides during cure had no significant effect on later operations (Table VII). The other treatments, i.e., washing and wiping, had a significant effect on the number of organisms growing on nutrient agar and 10% salt agar.

Statistical methods were employed to determine whether washing or wiping had the greater effect. Table VIII shows that wiping, but not washing, had a significant effect on the number of organisms growing on all media.

Since the lower number of organisms on the sides after wiping was reflected in a proportionately lower number after storage (Fig. 5), it may be assumed that the growth rate during the storage period was approximately the same on wiped and unwiped sides. Wiping, as practised in this investigation, should therefore lengthen the slime-free life of the sides (5). An analysis of variance of the logarithm of the total number of organisms on the sides at

TABLE VIII

ANALYSIS OF VARIANCE OF TOTAL DIFFERENCES IN LOGARITHM OF SURFACE COUNT OF WILTSHIRE SIDES TO SHOW EFFECT OF TREATMENTS, OTHER THAN SALTING, DURING CURE

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Washing	1	0.47	0.71	0.49
Wiping	1	28.8 **	19.8 *	26.5 **
Interaction	1	0.47	0.90	1.65
Error	8	1.44	1.85	1.29

* Indicates 5% level of significance.

** Indicates 1% level of significance.

the end of storage showed that wiping was the only treatment that had a significant effect on the final number of bacteria present (Table IX). Neither washing nor salting as carried out in this investigation had a beneficial action.

TABLE IX

ANALYSIS OF VARIANCE OF LOGARITHM OF TOTAL NUMBER OF ORGANISMS ON SURFACE OF WILTSHIRE SIDES AFTER STORAGE

Variance attributable to	D.f.	Mean square		
		Nut. agar	4% salt agar	10% salt agar
Salting	2	1.28	0.91	0.95
Wiped—not wiped	1	21.8 *	31.4 *	34.8 *
Other treatment	2	0.68	0.51	0.43
Error	6	3.36	5.23	3.11

* Indicates 5% level of significance.

Discussion

Previous work suggested that the number of bacteria increased in the pickle during the cure of Wiltshire sides (4). The results of the present investigation indicate that in the plant in which the tests were made such changes are small, if they occur at all. This is not considered to be a discrepancy, but attributable to the differences in sampling and the method of handling the samples in the plant and under the carefully controlled conditions of these studies. Since there was a considerable increase in the number of organisms on the sides, it is concluded that any changes attributable to bacterial action in the conversion of pork to bacon occur at the meat-pickle interface.

In a recent report of a few preliminary experiments (1), it was found that the number of organisms in pickle increased during cure, and that a reduction or very slight increase occurred on sides. However, since in those experiments the bacterial content was purposely reduced to a minimum, the results are not strictly comparable with those on commercial cures presented here.

Although no statistical computations were made, there is apparently little relation between the changes in bacterial and nitrate or nitrite content of the pickle during cure. This does not preclude the existence of such a relation, however, since the formation of nitrite from nitrate as a result of bacterial action may be masked by its reaction and diffusion into the tissue, or its reduction to other nitrogen compounds.

Acknowledgments

The authors wish to express their thanks to Messrs. W. G. McLeod and W. J. Hodder of Canada Packers Ltd., for their co-operation, to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for assistance with the statistical computations, and to Messrs. A. Morrison and S. G. Harbottle, Laboratory Assistants, for technical assistance.

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INTERACTION BETWEEN THE AUTOSOMES OF *DROSOPHILA MELANOGASTER* AS MEASURED BY VIABILITY AND RATE OF DEVELOPMENT¹

By S. C. REED²

Abstract

Females of an inbred *al c : se ss : ey^R* mutant strain were crossed with males from a "wild" strain that had been inbred (brother \times sister) for more than 60 generations. The F_1 males were mated with mutant strain females. The back-cross offspring would be expected to appear in eight genotypes with equal frequencies were there no differences in viability. The marker genes permitted the scoring of each fly as to whether it was heterozygous or homozygous for each of the mutant autosomes.

The genes used as markers probably had a distinctly negative effect upon both viability and rate of development, but the second pair of autosomes in combination with the other mutant autosomes increased viability, when homozygous, to a greater extent than did a heterozygous mutant-wild type pair. Apparently the possible negative effects of the markers *al* and *c* were more than offset by other genes on this autosome which had positive effects. The negative effects of the homozygous mutant third and fourth chromosomes were severe when in combination.

The interactions of the positive second chromosome and the negative third and fourth chromosomes in the eight genotypes were of some geometric order. It was found that the addition of a "positive" or "negative" autosome to any genotype caused a change in viability in the direction of the added autosome, but the *amount* of change is at present unpredictable and depends upon the particular combination to which the autosome was added.

The relation between the different autosomes and the rate of development was quite different from the relation between autosomes and viability. The second chromosome (marked by *al c*), which gave the only positive contribution to viability, retarded development more than either the third (*se ss*) or the fourth (*ey^R*). The effects of the three autosomes on rate of development were not strictly additive.

It is concluded that there is interaction of the genes for quantitative characters and this interaction is geometric in nature. The significance of the results in their relation to some theories of the inheritance of quantitative characters is discussed.

Most of the investigators interested in the inheritance of quantitative characters have chosen to study the inheritance of size. East and Castle were pioneers in the study of size inheritance, but Sax (6) was the first to present a good demonstration of an association between qualitative and quantitative factors. Later MacArthur and Butler (3) provided clear evidence that genes for size of tomato fruits react in a geometric fashion. Charles and Smith (1) came to the same conclusion regarding gene interaction in tomatoes and tobacco and published useful algebraic formulae that enable one to distinguish between arithmetic and geometric gene action from a study of the means, variances, and third moments of the F_2 and other generations.

¹ Manuscript received September 9, 1940.

Contribution from the Department of Genetics, McGill University, Montreal, Que. Research supported by a grant for genetics and cytology from the Rockefeller Foundation to McGill University.

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Rasmusson's (5) theory of genic interaction assumes that the total effect of the genotype is not determined by the direct simple addition of the effects of quantitative genes, but that they interact with one another in such a way that the cumulative effect of all the genes is less than the sum of their individual effects; the distributions should be negatively skewed according to this conception. Powers (4) in his work on barley obtained data that support the view that the cumulative effect of a number of genes is greater than the sum of their individual effects. This hypothesis calls for positive skewness of the distributions. Sinnott (7) found, in experiments with *Cucurbita*, that 41 out of 43 F_2 populations had definite positive skewness. An F_2 distribution that forms a normal curve is assumed to show the absence of gene interaction.

These views of the relation between curve shape and type of factor interaction are rather an over-simplification because, as Charles and Smith (1) have shown, positive skewness is an indication of geometric factor effects only if large and small parent strains have equal non-genetic variances. If the larger strain has a larger standard deviation, as is perhaps more commonly the case, some positive skewness is thereby introduced into the F_2 , whatever the type of factor interaction.

Because *Drosophila* is so valuable for the study of qualitative characters and such poor material for the study of size inheritance remarkably little work has been done on quantitative inheritance in this species. However, many characters of a quantitative nature, other than size, could be studied profitably in *Drosophila*. Timofeeff-Ressovsky (8) was one of the many investigators aware of the great advantages that *Drosophila* presents for a study of some quantitative characters and accordingly studied the effect on vitality of six sex-linked genes in *Drosophila funebris*. It was found that the gene "eversae" increased vitality relative to the normal allele, but that the other five mutant genes decreased vitality. The crosses were between isogenic strains and allowed exact calculation of the effects and interactions of each of the six mutations. Timofeeff-Ressovsky found large deviations of the observed from the expected values on a strictly additive basis. Thus there were interactions between the genes but the interactions followed no uniform rule. Each combination of genes produced a new and unrelated vitality threshold. The work with *Drosophila funebris* was concerned with the effects of six mutations all located on the X chromosome. The material to be presented here concerns the effects of the three autosomes of *Drosophila melanogaster*. Whereas Timofeeff-Ressovsky demonstrated the type of reactions that may be expected from mutant genes all of which are within the same chromosome, the present work gives a clear description of the interactions between whole chromosomes. The types of interactions for two distinct quantitative characters, viability and rate of development, will be demonstrated. The absence of crossing-over between any of the chromosomes involved in the generations studied allows an analysis of chromosome interactions which would not be feasible with plant and most animal material. In this paper the term "viability" has been used rather than Timofeeff-

Ressovsky's "vitality". The meaning of both terms is the same here, i.e., the production of an adult fly from the fertilized egg. The duration of life has not been considered in these experiments; data concerned with that problem were published by Gonzalez (2).

Methods

Females of an inbred strain that bore markers on the three pairs of autosomes were crossed with males of a "wild" type strain that had been inbred for more than 60 generations. The F_1 males were of the "wild" phenotype but were heterozygous for each of the autosomes from the mutant strain mother. This mutant strain was generally known as the "C" stock and possessed the marker genes, aristaless (*al*) and curved (*c*) on the second chromosome. The third chromosome of the mutant race was marked with the genes sepia (*se*) and spineless (*ss*), and the fourth chromosome carried the marker gene eyeless (*ey^R*). The F_1 males, in which, of course, there was no crossing-over, were mated with females of the mutant "C" stock to produce a backcross generation. The backcross progeny all derived their sex chromosomes from the "C" stock but were homozygous or heterozygous for the "C" stock genes on the autosomes. The marker genes allow scoring the backcross flies for the racial origin of each autosome that had the precise genic composition of the race from which it was originally derived.

Eggs from the mating of the pure "C" stock females with the F_1 males were deposited in large numbers upon corn meal agar in Petri dishes. The eggs were removed from the Petri dishes a few hours after they were laid and placed in large vials, 20 eggs in each vial. The vials contained "slants" of corn meal agar lightly sprayed with yeast one day before use. The flies appearing in each vial were scored for sex and autosomal constitution within 12 hr. after emergence from the pupa case. If all classes were equally viable, they should fall into eight different genetic classes with equal frequencies or, with the sexes treated separately, into sixteen classes.

Crude methods were used in studying the differences in the rates of development of the different genotypes. A very accurate method involving continuous observation of each egg was developed but, as the number of eggs that could be followed at one time was small, the crude method, which allowed observations in large numbers, was more valuable for this particular problem. The crude method consisted simply of recording the order in which flies of the various genotypes emerged from their pupa cases. If it were impossible to tell which fly in a vial was the first or the last to emerge, that vial was discarded in the study of the rate of development. It is realized that the eggs when laid were not all in the same stage of development.

The eggs and flies were always kept in an incubator held at $24^\circ \pm 0.5^\circ \text{C}$. The incubator was large enough to accommodate the investigator. All observations were made by the author himself and this should have tended to reduce experimental error.

Examination of the salivary chromosomes of the strains showed them to be apparently normal. Certainly they had no large inversions or deficiencies.

Experimental Data

Viability

The viability of the parental strains and backcross flies is shown in Table I. The difference in viability between the wild inbred stock and the mutant inbred stock is very great, amounting to 54.4%. The viability of the F_1 male genotype (in "C" cytoplasm) and of the whole backcross generation is approximately intermediate between that of the pure strains, though nearer that of the "C" stock. It is clear that though all the marker genes were completely recessive with regard to visible characters, the effects of the whole autosomes on viability of the F_1 gave intermediacy or less. The genes concerned with the difference in viability between the two strains collectively, but not necessarily individually, show lack of dominance such as is often associated with quantitative characters.

TABLE I

VIABILITY OF PARENTAL STRAIN, OF F_1 MALES, AND THE BACKCROSS GENERATION AS A WHOLE

Strain	Number of eggs	Number of flies emerged			Viability, %
		♀ ♀	♂ ♂	Total	
Wild	2000	735	751	1486	74.1
F_1	1130	230	214	444	39.3
Backcross to "C" stock	5500	1124	1165	2289	41.6
Mutant "C"	1500	122	174	296	19.7

The results for each genotype of the backcross generation are shown in Table II. It will be seen that only one of the markers on each of the second and third chromosomes is listed in the genotype. This is merely a convenient abbreviation; the other markers were also present, of course.

There is clearly a differential viability of the different genotypes but no statistically significant difference between the viability of the wild type heterozygote $\left\{ \frac{+}{c} \frac{+}{se} \frac{+}{ey} \right\}$ and the flies homozygous for the second autosome $\left\{ \frac{c}{c} \frac{+}{se} \frac{+}{ey} \right\}$ or those homozygous for the third autosome $\left\{ \frac{+}{c} \frac{+}{se} \frac{ey}{ey} \right\}$. The decrease in viability when the *ey* gene is homozygous $\left\{ \frac{+}{c} \frac{+}{se} \frac{ey}{ey} \right\}$ is highly significant. It is very probable that the decrease in viability may be attributed to the action of the *ey* gene itself, particularly as *ey* disturbs the morphology of the head so markedly.

The sepia eyeless flies with the genotype $\left\{ \frac{+}{c} \frac{se}{se} \frac{ey}{ey} \right\}$ have the lowest viability. Their viability is significantly lower than that of the class of flies

that is genetically identical with the homozygous "C" stock $\left\{ \frac{c}{c} \frac{se}{se} \frac{ey}{ey} \right\}$. This is perhaps surprising because segregates homozygous for the three mutant autosomes might be expected to have a lower viability than segregates homozygous for only two mutant autosomes. An explanation for this appears when it is noted that homozygosity for the autosomes marked by the "curved" gene raised viability in two different combinations of the mutants (Table III). Considering all the combinations, it is found that the "curved" chromosome increased viability in two combinations, left it unchanged in one, and decreased it in the fourth; the third chromosome decreased viability in three combinations and left it unchanged in one, whereas the fourth decreased viability in all four combinations. The increase in viability resulting from the addition of the homozygous "curved" chromosome is highly significant

TABLE II

RELATIVE VIABILITY OF THE DIFFERENT GENOTYPES OF THE BACKCROSS GENERATION. OF THE TOTAL OF 5500 EGGS STARTED ABOUT 688 SHOULD BE EXPECTED TO BE OF EACH OF THE EIGHT GENOTYPES*

Genotype	First experiment	Second experiment	Third experiment	Totals	Viability of the different genotypes relative to $\frac{+}{c} \frac{se}{se} \frac{+}{ey}$, %
$\frac{+}{c} \frac{+}{se} \frac{+}{ey}$	72	66	221	359	95.5
$\frac{+}{c} \frac{se}{se} \frac{+}{ey}$	76	62	238	376	100.0
$\frac{c}{c} \frac{+}{se} \frac{+}{ey}$	75	74	205	354	94.2
$\frac{+}{c} \frac{+}{se} \frac{ey}{ey}$	55	30	163	248	66.0
$\frac{c}{c} \frac{+}{se} \frac{ey}{ey}$	63	66	203	332	88.3
$\frac{c}{c} \frac{se}{se} \frac{+}{ey}$	53	45	195	293	77.9
$\frac{+}{c} \frac{se}{se} \frac{ey}{ey}$	24	13	80	117	31.2
$\frac{c}{c} \frac{se}{se} \frac{ey}{ey}$	40	34	136	210	55.8
	458	390	1441	2289	

*The sexes have been treated separately but as there were no significant differences between them the data are not included here.

TABLE III

THE MODIFICATION OF VIABILITY RESULTING FROM VARIOUS COMBINATIONS OF THE HOMOZYGOUS MUTANT AUTOSOMES. IN COMBINATION THE CURVED ($\frac{c}{c}$) AUTOSOME ALWAYS INCREASES VIABILITY BUT SEPIA AND EYELESS AUTOSOMES ALWAYS DECREASE VIABILITY.

Mutant autosome combination	Effect on viability, %	Mutant autosome combination	Effect on viability, %	Mutant autosome combination	Effect on viability, %
$\frac{c}{c}$ with normal	Unchanged	$\frac{se}{se}$ with normal	Unchanged	$\frac{ey}{ey}$ with normal	Decreased 34.0
$\frac{c}{c}$ with $\frac{ey}{ey}$	Increased 33.8	$\frac{se}{se}$ with $\frac{ey}{ey}$	Decreased 52.8	$\frac{ey}{ey}$ with $\frac{se}{se}$	Decreased 68.9
$\frac{c}{c}$ with $\frac{se}{se} \frac{ey}{ey}$	Increased 79.4	$\frac{se}{se}$ with $\frac{c}{c} \frac{ey}{ey}$	Decreased 36.7	$\frac{ey}{ey}$ with $\frac{c}{c} \frac{se}{se}$	Decreased 28.3
$\frac{c}{c}$ with $\frac{se}{se}$	Decreased 22.1	$\frac{se}{se}$ with $\frac{c}{c}$	Decreased 17.2	$\frac{ey}{ey}$ with $\frac{c}{c}$	Decreased 6.2

and must be considered real. The general trend seems to be one in which the "curved" autosome *increases* viability most when in combination with autosomes that otherwise *decrease* it most. Both the "sepia" and "eyeless" autosomes seem to *decrease* viability to the greatest extent when added to combinations that do likewise. In other words, the lower the viability of a combination, the greater is the *positive* effect if the "curved" autosome is added. But the lower the viability of a genotype, the greater is the effect, in a negative direction, that results when another deleterious autosome in the homozygous condition is added to the combination. The increase in effect is clearly not arithmetic but is a geometric or power function of some sort. Apparently the further from the normal a genotype may be, the more sensitive it is to the addition of a given autosome and the greater the effect on the phenotype. The addition of a positive or negative autosome to any genotype will cause a change in viability in the *direction* of the added autosome, but the *amount* of change is at present unpredictable and depends upon the particular combination to which the autosome was added. Furthermore it should not be considered extraordinary for an autosome to give a positive effect in one combination and a negative effect in some other combination.

Rate of Development

It became apparent after the synthesis of the mutant "C" stock that the rate of development of the "C" flies was slower than that of the "wild" stock. By the crude method of observing the length of time from the laying of the eggs to the emergence of the flies, were obtained the observations on length of the developmental period shown in Table IV. The differences between the average lengths of time taken for development are all statistically significant. At 25° C., the mutant "C" stock took about ten days for development in comparison with nine days for the "wild" stock. Development of the backcross generation covered a wider range of time than the "wild" stock, the average period thus being slightly less. The backcross generation

TABLE IV
DURATION OF DEVELOPMENTAL PERIOD OF THE PARENT STRAINS AND OF THE BACKCROSS GENERATION

Strain	Length of developmental period, days								Total number of flies	Average developmental period, days
	7	8	9	10	11	12	13	14		
	Number of flies									
"Wild"	0	76	1213	180	13	4			1486	9.09 ± 0.01
Backcross generation	41	810	1100	263	67	8			2289	8.79 ± 0.02
"C" stock	0	17	52	168	47	5	4	3	296	9.98 ± 0.05

seemed to give a small display of heterosis, as demonstrated by the faster rate of development.

An inspection of the rates of development of the different genotypes of the backcross generation is of considerable value. Table V shows the percentage of flies of each genotype to emerge first and last. The genotype of the first and of the last fly to emerge in each vial was recorded. The data

TABLE V
THE PERCENTAGE OF FLIES OF EACH GENOTYPE TO EMERGE FIRST AND LAST IN EACH VIAL

Genotype	First fly to emerge		Last fly to emerge	
	No. of cases	%	No. of cases	%
$\frac{+}{c} \frac{+}{se} \frac{+}{ey}$	64	17.8	3	0.8
$\frac{+}{c} \frac{+}{se} \frac{ey}{ey}$	26	10.5	8	3.2
$\frac{+}{c} \frac{se}{se} \frac{+}{ey}$	35	9.3	17	4.5
$\frac{c}{c} \frac{+}{se} \frac{+}{ey}$	26	7.4	18	5.1
$\frac{+}{c} \frac{se}{se} \frac{ey}{ey}$	6	5.1	8	6.8
$\frac{c}{c} \frac{+}{se} \frac{ey}{ey}$	13	3.9	23	6.9
$\frac{c}{c} \frac{se}{se} \frac{+}{ey}$	8	2.7	49	16.7
$\frac{c}{c} \frac{se}{se} \frac{ey}{ey}$	5	2.4	46	21.9

so obtained are presented in the table. If more than one fly had emerged when observations on a particular vial were made, that vial had to be disregarded for this part of the problem. The evidence from the table is very clear cut. The "wild" type flies emerged first, those homozygous for the "eyeless" autosome next, those homozygous for the "sepia" autosome third, and finally, those homozygous for the "curved" autosome. Flies having these combinations of the autosomes emerged in the order expected from the observations on the retardation of development by the single mutant autosomes. Of the group of flies, in which each was the last in its vial to emerge, the genotypes were again in the order that shows the greatest retardation by the "curved" autosome, next by the "sepia" autosome, and least retardation by the "eyeless" autosome. This is interesting, for the effects on development were precisely the opposite of the effects of the same autosomes on viability. Whereas the "eyeless" autosome reduced viability most, it retarded development least; the curved autosome actually increased viability but at the same time retarded development.

From Table V the expected percentages of the combination genotypes according to various arithmetic additive schemes may be calculated and compared with the observed. This was done but the deviations were too great to allow the consideration that the effects of these autosomes on rate of development were strictly arithmetic. The effects on rate of development fitted more closely an arithmetic additive scheme than did the results on viability, but the deviations were too great in either case to allow the acceptance of such an interpretation. As with viability, it is clear that there was interaction of some geometric kind between the autosomes. It may be predicted that the addition of any one of the homozygous mutant autosomes to any combination of the others will retard development but it is not possible to predict the amount of retardation except for each genotype by itself.

It is of some interest to note that whereas the autosome bearing the mutant gene "eyeless" was the only one that, when homozygous, decreased viability without the aid of one of the other mutant autosomes, each of the three mutant autosomes retarded development without the aid of either of the others.

Discussion

As Powers (4) has pointed out, the nature of the interaction of factors affecting quantitative characters is sufficiently variable to require extensive genetic studies involving a large variety of characters and organisms before an hypothesis of much value for prediction purposes can be formulated. Some of the most valuable experiments for such a formulation, for example, the experiment of Timofeeff-Ressovsky of 1935, may be only indirectly related to the subject. Timofeeff-Ressovsky's work was a demonstration of the production by the use of X-rays of mutations affecting vitality. The induced mutations had no observable effects other than their influence upon vitality. These small physiological mutations were shown to be hereditary and though most of them decreased vitality, one case was discovered in which vitality

was improved. The vitality mutations were more frequent than other types and the beneficial ones might be preserved now and again through the action of Natural Selection.

Whereas Timofeeff-Ressovsky (8) studied the interactions between specific genes on a single chromosome and found that two genes working together followed no uniform rule, the author studied interactions between all the autosomes of *Drosophila* at once. It was found, as might be expected, that the amount of change produced by an autosome depended upon the rest of the combination and, because this amount varied for each combination, it is clear that interaction was present and that it was not a simple case of addition of constant values.

It was found further that the most aberrant genotypes were affected to the greatest degree by the addition of an autosome having positive or negative effects on viability. That is, the less normal a genotype, the more sensitive it was to the influence of an autosome affecting viability in either a positive or negative direction. This is another type of illustration of Powers' (4) conception that the cumulative effect of a number of genes is *greater* than the sum of their individual effects.

At first glance this would seem a direct contradiction of the formulation by Rasmusson (5) that quantitative characters have a definite upper limit which, because of mechanical or other reasons, cannot be exceeded. In other words, the more closely the limit is approached by the addition of advantageous genes, the smaller is the effect of each gene; therefore the cumulative effect of all of them becomes less than the sum of their individual effects. The hypothesis of Powers' might be called one of "compound interest increments" and that of Rasmusson one of "diminishing returns". However, since each conception is a picture of the same sort of facts read from a different angle, there is no contradiction. If the effects are read in one direction, the diminishing returns effect is obtained, in the other, the compound interest effect. The case of the addition of the "curved" gene may be considered. Its effect on viability is less and less beneficial, the better the viability of the rest of the genotype; this is a case of "diminishing returns". Looked at from the other direction, the effect becomes greater the poorer the viability of the rest of the genotype—a case of "compound interest". In any allelic pair, one member increases viability and the other decreases it, against a given genetic background. Naturally, the structure and genotype of the organism produce a limit at both ends of the scale of these effects.

In general it may be stated that there is an interaction of the genes for quantitative characters and this interaction is geometric in nature. Various schemes were designed to discover the precise nature of the geometric interaction but none of them were entirely satisfactory. It is perhaps best to report this system of geometric interactions without attempting, at present, a practical scheme that would be weakened by the weight of necessary theoretical assumptions.

Acknowledgment

The author is grateful to Dr. D. R. Charles for criticisms and suggestions.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

MARCH, 1941

NUMBER 3

CANADIAN WILTSHIRE BACON

XVI. COLOUR AND COLOUR STABILITY OF PORK AFTER FROZEN STORAGE AND CONVERSION TO BACON¹

By W. H. COOK²

Abstract

Temperatures below -17.7°C . (0°F .) and moisture resistant, preferably impermeable, wrappings are desirable for the retention of colour of pork during storage. Although pork thawed in air or water was lighter in colour than that thawed in brine or pickle, the difference was markedly reduced after curing and maturation. Nevertheless, the colour of the final product was significantly correlated with that of the pork after thawing. Samples stored under conditions that retain the original colour, or subsequently thawed by procedures that produce light colours, are the least stable after defrosting. On the average there was comparatively little difference between the colour of exposed and internal samples, although the former were more variable. Pale-drying yielded samples with a lighter internal colour than did the usual smoking procedure.

Introduction

In Canada it is frequently necessary to store appreciable quantities of frozen pork for subsequent use in the manufacture of bacon. Storage conditions that yield a product suitable for immediate consumption as pork may not be suitable for the production of bacon of acceptable quality, since certain changes that occur to only a minor extent during storage may be exaggerated during thawing, curing, or maturing. In a previous paper (2) on the frozen storage of pork, it was shown that storage temperature had a significant effect on the colour of the lean and the rancidity in the fat. Nevertheless, within the temperature range commonly employed for the storage of pork for comparable periods in commercial practice (-15°C . or lower) these changes were too small to have any serious effect on the quality of the pork for immediate consumption. The investigation reported here was undertaken to determine the effects of various storage conditions and thawing procedures on the quality of the product at all stages of processing, including the matured bacon.

Colour and colour stability of the lean, and the peroxide oxygen and free fatty acid content of the fats, were used as criteria of quality, since changes in bloom caused by drying, and changes in the pigments and fats due to oxidation are among the first storage defects to become evident. The results of the fat analyses will be reserved for a later paper.

¹ Manuscript received November 16, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as paper No. 58 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 980.

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Material and Methods

The material, which consisted of full-length rib-in pork backs, was subjected to various storage conditions. Duplicate samples were allotted at random to the various sets of conditions, giving a total of 30 pieces. The storage temperatures were -6.6° , -12.2° , -17.7° , -23.4° , and -29.0° C. (20° , 10° , 0° , -10° , and -20° F.). Since it was difficult to provide precise control of relative humidity for these large cuts, three wrapping practices, with wrappers graded to represent various degrees of permeability to water vapour, were substituted for controlled humidities at each storage temperature. This procedure emulates commercial practice where wrapping is used to reduce surface drying. The wrappings used were as follows: single layer of waxed glassine paper, adequately overwrapped and tied but not sealed; two layers of waxed glassine, each layer applied and tied separately as above, and not sealed; and aluminium foil solidly laminated to sulphite paper and sealed. All the packages were protected against mechanical damage during storage by overwrapping with a single layer of heavy Kraft paper.

TABLE I
TREATMENT OF SAMPLES AFTER REMOVAL FROM STORAGE

Stage	Method	Temperature, $^{\circ}$ C.	Time, hr.	Additional exposure after sampling
Thawing	In air (wrapped)	10.0	25	6 hr. in air at 7.5° C.
	In brine	4.5	5	2 hr. in air at 7.5° C.
	In pickle	4.5	4½	2 hr. in air at 7.5° C.
	In water	38.0	1½	1½ hr. in air at 7.5° C.
Cure	Wiltshire pickle	3.6	88	Sampled on removal
Maturation	In air (covered)	3.2	168	Sampled on removal
Subsequent treatment	Smoked in commercial smoke house	55	7½	Sampled for colour on following day, and for fat analyses five days later. Temp. 10° C.
	Heated in laboratory oven	55	7½	

After 48 weeks' storage under these conditions, the material was examined, and each piece cut into two approximately equal portions. The four pieces thus obtained from each storage treatment were allotted at random to the four thawing procedures described in Table I. The exposure period was that required to bring the internal temperature of the samples to a curing temperature of 3.6° C. (38° F.). The brine and pickle were maintained at the specified temperatures with electrical heaters, and the water by the addition of steam.

All samples were cured together in a commercial Wiltshire pickle (Table I), obtained from a packing plant. Pumping was considered unnecessary for these comparatively small pieces, and the curing period was reduced to less

than four days in an attempt to approximate the salt content obtained commercially. Analysis of four samples, taken at random from each of the four groups of thawing procedures, after curing and smoking or pale-drying, showed average chloride, nitrate, and nitrite contents of 5.93, 0.039, and 0.003%, respectively. Those thawed in pickle had a somewhat higher chloride content (6.57%) than those thawed by the other procedures, but there were no other systematic differences. These values are well within the range of those obtained in commercially cured and smoked samples (1, 5).

The material was sampled after thawing, cure, and again after a 7-day maturation period. This was the last systematic sampling that represented all storage treatments and thawing procedures. The 60 pieces remaining as residues were then divided at random into two groups of 30; one group was smoked in a commercial smokehouse, and the other pale-dried by heating at the same temperature under similar temperature conditions for a comparable period in a large laboratory oven. Measurements were made on these two groups to compare the effect of smoking and pale-drying.

The colour measurements were made by a procedure already described (3). Colour stability was estimated by repeating the colour measurements after the samples had been exposed for 72 hr. at 10° C. and 95% relative humidity.

Colour Measurements on Internal Surfaces

In order to reduce the data, only the average values for each condition and an analysis of variance on the detailed results are reported (Tables II and III). It is evident from the latter that each of the four primary factors (storage temperature, wrapping, thawing, and stage of conversion) had significant effects on each of the colour components. In addition the factors frequently showed significant differential effects. Since the direct effect of a given condition seldom significantly exceeded the differential effects, the tests of statistical significance were made by comparison with the residual variance or experimental error.

Since the scatter of the several colour components are correlated (4) only the total of all scatters, or brightness, will be discussed. The colours of the samples stored at -6.6° and -29° C. were significantly brighter than those stored at the intermediate temperatures. The increased brightness at -6.6° C. is due to methaemoglobin formation, whereas that observed at -29° C. approximates the value for fresh pork (2). Since the brightness at the three intermediate temperatures was below normal it is concluded that some drying occurred. At -12.2° C. this was possibly offset by slight methaemoglobin formation, although no visible effects were evident.

The differences observed between the various wrapping procedures were as expected. The brightness increased significantly in order of increasing impermeability of the wrapper to water vapour, showing the effect of differential evaporation from the stored product.

TABLE II
COLOUR OF PORK STORED AND CURED UNDER VARIOUS CONDITIONS

Colour component	Average scatter at each storage temperature					
	-6.6° C.	-12.2° C.	-17.7° C.	-23.4° C.	-29.0° C.	Necessary difference
Blue	12.9	11.6	11.8	11.2	12.2	0.70
Green	17.0	15.1	15.2	14.5	16.1	0.96
Red	27.4	24.8	24.5	23.8	26.2	1.21
Total (Brightness)	57.2	51.5	51.4	49.6	54.4	2.77
	Average scatter for each method of wrapping					Necessary difference
	Single wrap, waxed paper	Double wrap, waxed paper	Single wrap, aluminium foil			
Blue	11.3	12.0	12.5	0.16		
Green	14.6	15.6	16.5	0.74		
Red	24.2	25.0	26.9	0.94		
Total (Brightness)	50.0	52.6	56.0	2.15		
	Average scatter for each method of thawing					Necessary difference
	In brine	In pickle	In air	In water		
Blue	11.4	11.6	12.2	12.5	0.59	
Green	14.6	14.8	16.1	16.6	0.86	
Red	23.8	23.8	26.6	27.1	1.08	
Total (Brightness)	49.8	50.3	55.0	56.2	2.48	
	Average scatter at each stage of conversion					Necessary difference
	After defrosting	After curing	After maturation			
Blue	13.3	10.8	11.7	0.16		
Green	18.3	13.6	14.7	0.74		
Red	28.3	23.6	24.2	0.94		
Total (Brightness)	60.0	48.0	50.6	2.15		

The thawing procedures fell into two groups with respect to their effect on colour. Immersion in brine or pickle produced samples that did not differ significantly from one another, but both yielded pork considerably darker in colour than that thawed in air or water. An adequate explanation for this behaviour cannot be given, but the withdrawal of water from the pork immersed in the salt solutions may be the responsible factor. A similar behaviour was observed during cure when all of the samples, regardless of the previous storage and defrosting treatment, darkened considerably. During subsequent maturation there was a comparatively small, but significant increase in brightness.

Since all of the primary factors affected the colour, differential effects were to be expected over the range of each condition studied. The analysis of variance shows that most of these interactions were significant, and of a magnitude comparable to the primary factors. Thus, on the average, wrap-

TABLE III
ANALYSIS OF VARIANCE OF COLOUR MEASUREMENTS

Variance due to:	D.f.	Mean square			
		Blue	Green	Red	Total (Brightness)
Temperature	4	13.9***	34.4***	74.8***	328 ***
Wrapping	2	23.2***	57.8***	122 ***	539 ***
Thawing	3	12.3***	43.9***	140 ***	476 ***
Stage of conversion	2	101 ***	367 ***	387 ***	2376 ***
Temperature X wrapping	8	13.6***	30.9***	53.4***	273 ***
Temperature X thawing	12	6.4***	17.1***	27.2***	140 ***
Temperature X stage	8	1.4	1.3	0.8	9.0
Wrapping X thawing	6	5.1*	12.0*	21.3**	96.8*
Wrapping X stage	4	0.8	1.3	1.2	9.1
Thawing X stage	6	13.3***	42.3***	149 ***	489 ***
Error	124	2.0	4.2	6.7	35.2

* Indicates 5% level of significance compared with residual.

** Indicates 1% level of significance compared with residual.

*** Indicates 0.1% level of significance compared with residual.

ping had significant effects, but the differences were smaller at low, than at high, storage temperatures. Similarly, pork stored under conditions that may tend toward the production of a lighter or darker colour may be affected differentially by the thawing procedure, those thawed in air or water being lighter than those immersed in brine or pickle. In this connection it is of interest to note that the stage of conversion of pork to bacon did not show differential effects with any of the factors except method of thawing. This implies that certain thawing procedures affect colour in the same manner as curing, whereas others do not. It is therefore of interest to examine the effects of defrosting and stage of conversion in greater detail.

Table IV shows the brightness at each stage for each method of defrosting, averaged for all storage treatments. Immediately after thawing in brine, the value of the colour is approximately the average for pork. By comparison, pickle causes slight darkening whereas, at this stage, the other two methods of thawing yield much lighter samples. During cure all of the samples darkened somewhat, the most marked change occurring in the bright samples that had been thawed in air or water. All have about the same brightness after cure. During maturation all samples became brighter, with those thawed in pickle showing the greatest increase.

The extent of correlation of the colours observed at the three stages was determined by computing simple coefficients of correlation. The coefficients of correlation between the brightness before and after cure were generally largest, and those between the values before and after maturation lowest (Table IV). These lower values are doubtless attributable to the smaller average change during maturation that rendered the experimental error

TABLE IV
EFFECT OF METHOD OF THAWING ON COLOUR (BRIGHTNESS) OF LEAN AT VARIOUS STAGES
DURING CURE

Thawed in	Total scatter, %			
	After thawing	After cure	After maturation	
Brine	53.8	46.7	48.9	
Pickle	49.9	47.5	53.7	
Air	67.0	48.6	49.3	
Water	69.1	49.2	50.4	
	Coefficients of correlation between total scatter at the various stages			
	Degrees freedom	After defrosting with after cure	After cure with after maturation	After defrosting with after maturation
Brine	13	0.84**	0.54*	0.64**
Pickle	13	0.91**	0.78**	0.6**
Air	13	0.65**	0.56*	0.64**
Water	13	0.70**	0.52*	0.57*
For all methods	58	0.63**	0.60**	0.37**

* Indicates 5% level of significance.

** Indicates 1% level of significance.

proportionately larger. The coefficients given in the last column of the table are of greatest interest since they show the extent to which the colour of the bacon depends on the colour of the thawed pork. These are of a magnitude sufficient to indicate a considerable degree of dependence, being greatest for material thawed in pickle (2).

Colour Stability Measurements on Internal Surfaces

The results of the colour stability measurements appear in Tables V and VI. A number of changes in the individual components did not attain significance presumably owing to the proportionately greater experimental error (3). Brightness stability, the only attribute of colour quality discussed here was usually affected significantly by each of the four factors under investigation. The effect of storage temperature and wrapping procedure, however, had less effect than the method of thawing and the stage of conversion. The effect of storage temperature and wrapping can be summarized by stating that a low storage temperature and wrapping in an impermeable wrapper, conditions that favour maintenance of the original colour during storage, yield material that suffered the most darkening during exposure. Reasons for this behaviour have already been given (12).

The effect of the method of thawing shows a similar behaviour. The lighter samples produced by thawing in air and water were least stable during exposure whereas the dark samples produced by thawing in brine and pickle showed little change. On the average, all samples darkened after thawing; after

TABLE V
COLOUR STABILITY OF PORK STORED AND CURED UNDER VARIOUS CONDITIONS

Colour component	Average change in scatter at each storage temperature					
	-6.6° C.	-12.2° C.	-17.7° C.	-23.4° C.	-29.0° C.	Necessary difference
Blue	+0.022	+0.111	-0.044	-0.183	-0.200	0.27
Green	-0.011	+0.211	+0.222	-0.092	-0.258	0.34
Red	-0.844	-0.964	-0.992	-1.04	-1.25	0.55
Total (Brightness)	-0.830	-0.647	-0.814	-1.32	-1.71	0.78
	Average change in scatter for each method of wrapping					Necessary difference
	Single wrap, waxed paper	Double wrap, waxed paper	Single wrap, aluminium foil			
Blue	-0.013	-0.052	-0.112		0.21	
Green	+0.192	+0.028	-0.177		0.26	
Red	-1.01	-0.768	-1.28		0.43	
Total (Brightness)	-0.832	-0.792	-1.57		0.61	
	Average change in scatter for each method of thawing					Necessary difference
	In brine	In pickle	In air	In water		
Blue	+0.244	+0.093	-0.217	-0.355	0.24	
Green	+0.638	+0.011	-0.180	-0.411	0.30	
Red	-0.702	-0.151	-1.75	-1.47	0.50	
Total (Brightness)	+0.135	-0.038	-1.6	-1.68	0.70	
	Average change in scatter at each stage of conversion					Necessary difference
	After defrosting	After curing	After maturation			
Blue	-0.368	-0.118	+0.310		0.21	
Green	-0.270	-0.220	+0.530		0.26	
Red	-2.14	-0.863	-0.050		0.43	
Total (Brightness)	-2.78	-1.20	+0.790		0.61	

curing they were more stable but still darkened significantly, whereas at the end of maturation they became significantly brighter.

The effects of thawing method, stage of conversion, and the interaction of these variables on colour stability is best shown from the detailed results appearing in Table VII. Most of the differences in brightness stability between the various individual treatments are too small to attain significance. Since the colour stability of samples thawed in air and water improved during cure and maturation, the method of thawing has little effect on the stability of the final product. Correlation coefficients computed between the colour stability at different stages were, with one exception, uniformly insignificant. For the air-thawed samples the correlation between colour stability after thawing and after cure exceeded the 5% level of significance ($r = 0.56$).

TABLE VI
ANALYSIS OF VARIANCE OF COLOUR STABILITY MEASUREMENTS

Variance due to:	Mean square				
	D.f.	Blue	Green	Red	Total (Brightness)
Temperature	4	0.64	1.51*	0.78	7.01*
Wrapping	2	0.15	2.04*	3.91	11.4 *
Thawing	3	3.43***	6.58***	23.8 ***	40.6 ***
Stage of development	2	7.06***	12.2 ***	66.6 ***	192 ***
Temperature × wrapping	8	0.45	0.97	2.43	8.25**
Temperature × thawing	12	0.53	1.97***	1.47	16.6 ***
Temperature × stage	8	1.10**	1.65**	0.44	7.15*
Wrapping × thawing	6	0.44	2.46***	0.90	22.0 ***
Wrapping × stage	4	0.46	0.56	0.35	2.52
Thawing × stage	6	3.46***	10.0 ***	27.3 ***	96.6 ***
Error	124	0.33	0.52	1.41	2.82

* Indicates 5% level of significance compared with residual.

** Indicates 1% level of significance compared with residual.

*** Indicates 0.1% level of significance compared with residual.

TABLE VII
EFFECT OF METHOD OF THAWING ON COLOUR STABILITY (BRIGHTNESS) OF LEAN AT VARIOUS STAGES DURING CURE

Thawed in	Change in total scatter, %		
	After thawing	After cure	After maturation
Brine	+0.85	-1.4	+1.1
Pickle	+0.29	-1.1	+0.66
Air	-6.8	-0.57	+0.91
Water	-5.5	-1.7	+0.50

Comparison of Colour of Internal and Exposed Surfaces

In commercial practice the colour of the exposed surface is frequently used to assess the suitability of a given storage or thawing practice. By making certain observations on the colour of these exposed surfaces it was possible to obtain a comparison of the colour on the internal and exposed surfaces of corresponding samples.

The 30 pieces subjected to the various storage treatments had 60 ends exposed during storage. These were divided at random into three groups of 20 for observation after thawing, cure, and maturation, respectively. This yielded surfaces that had been exposed to two or more of the successive processes studied. It was also possible to obtain surfaces, from the corresponding pieces, that had been exposed only to the process immediately preceding the stage at which the measurement was made, i.e., thawing, curing, and maturation or combinations of these factors.

TABLE VIII
DIFFERENCE BETWEEN COLOUR SCATTERS OF EXPOSED AND INTERNAL SURFACES AT
VARIOUS STAGES

Stage compared	Surfaces compared†	Difference between mean scatters				Remarks
		Blue	Green	Red	Total	
After thawing	I—Ss.t.	1.62 *	0.835	2.01	4.46	Both surfaces showed similar variability.
After cure	I—Sc.	-1.23 *	-1.76	-1.15	-4.13	Both surfaces showed similar variability.
	Sc—Ss.t.c.	0.875	-0.800	0.210	0.285	Surface colour more variable than internal colour in red and total only.
	I—Ss.t.c.	-0.350	-2.56 **	-0.935	-3.84	
After maturation	I—Sm.	0.130	0.030	-0.380	-0.220	Sm. more variable than I in blue only.
	Sm—Ss.t.c.m.	0.095	-0.655	0.780	0.225	Ss.t.c.m. more variable than Sm. in green only.
	I—Ss.t.c.m.	0.225	-0.625	0.405	0.005	Ss.t.c.m. more variable than I in all colour components.

† I = Percentage scatter from internal surface; S = Percentage scatter from exposed surface, for all treatments indicated by the subscripts, where s = storage, t = thawing, c = cure, and m = maturation.

* Indicates 5% level of significance.

** Indicates 1% level of significance.

These results are presented in Table VIII as the differences between the scatter of the internal surfaces minus that of the exposed surfaces. For comparisons not involving the internal colour, the scatter for the surface suffering the greatest exposure was subtracted from that of the surface having the least exposure. All differences are algebraic, negative values indicating that the exposed surface was brighter. Statistical methods were used to determine whether the observed differences exceeded the necessary difference computed for the variations within each group of 20 samples, taken at each stage.

It is evident from the table that comparatively few of the differences between the exposed and internal surfaces were statistically significant. Since each group of comparable samples was taken at random from material that had been treated by procedures that had affected the colour significantly, it is obvious that the difference between the internal and external surfaces would have to be large to attain significance. Nevertheless material showing similar variability may be used commercially, and it is of interest to determine whether exposure causes significant changes on such material.

Of the significant differences, exposure during storage and thawing reduced the blue scatter, whereas exposure during cure alone increased the scatter of this component. Surfaces exposed to storage, thawing, and cure showed an increased green scatter over that of the internal surface. The variability,

within groups of internal and exposed surfaces, was comparable until after curing, but subsequently the exposed samples were consistently more variable in one or more of the colour components.

Comparison of Colour and Colour Stability of Smoked and Pale-Dried Bacon

The results of the colour and colour stability measurements on the smoked and pale-dried residues from the previous treatments appear in Table IX. In all instances the pale-dried material was brighter than the smoked material. The two groups of samples did not differ, however, in their average colour stability. Smoking deposits material on the surface of the bacon, but it is difficult to see why this should affect the internal colour of the lean. It is possible that the colour differences were due to temperature effects for although the air temperature and time of exposure were similar for both groups the temperature attained by the samples may have differed owing to the obvious difference in the size of the smoking room and a laboratory oven. Further investigations of this point are under way.

TABLE IX

COMPARISON OF COLOUR AND COLOUR STABILITY OF SMOKED AND PALE-DRIED BACON

Colour measurement	Treatment	Internal colour	Internal colour after exposure 72 hr. at 50° F.	Colour stability
Blue	Smoked	14.58	13.92	-0.65
	Pale-dried	16.50	15.63	-0.87
	Difference	1.92**	1.71**	0.22
Green	Smoked	17.99	17.41	-0.59
	Pale-dried	20.14	19.49	-0.65
	Difference	2.15**	2.08*	0.06
Red	Smoked	28.02	27.21	-0.81
	Pale-dried	31.03	30.03	-0.99
	Difference	3.01**	2.82**	0.18
Total	Smoked	60.59	58.54	-2.05
	Pale-dried	67.68	65.16	-2.52
	Difference	7.09	6.62	0.47

* Indicates 5% level of significance.

** Indicates 1% level of significance.

Conclusions

The combination of wrapping in an impermeable sealed wrapper and low storage temperature (-29°C.) appears from these results to be ideal for the preservation of the internal colour of pork for storage periods of about one year. Nevertheless the colour changes under other conditions are frequently only small, and storage temperatures of -18°C. with a wrapping, reasonably

moisture resistant, should yield satisfactory results under commercial conditions.

The colour of the pork is significantly altered by the method of thawing. Thawing in brine or pickle produces darker samples than in air or water. Most of the differences observed after thawing, decrease markedly during cure and maturation, so that the final cured product is not seriously affected by the procedure used. Nevertheless thawing in pickle or brine appears to cause colour changes of the same nature that take place during cure. The greater colour permanence at all stages following thawing in pickle suggests that this method is preferable.

The effect of the various treatments on colour stability depends primarily on the effect of these treatments on the initial colour. Under storage conditions that preserve the original colour, and subsequent treatments that yield bright or light coloured samples the product generally shows the greatest instability on exposure. Concerning colour stability in the final matured bacon there is little to choose between pork thawed by the several procedures tested.

There was seldom any significant difference between the colour of the interior of the samples and that of the outside surfaces subjected to one or more of the treatments under investigation. This is attributable in part to the variation in the colour of the pieces following the various treatments. The exposed surfaces, however, were more variable than the interior after curing. Pale-dried bacon was lighter in colour than smoked bacon, but there was no significant difference in their colour stabilities.

Acknowledgments

The author wishes to thank Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice, and to acknowledge the assistance of Messrs. A. E. Chadderton and E. A. Rooke, Laboratory Assistants.

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CANADIAN WILTSHIRE BACON

XVII. RANCIDITY IN PORK FAT AFTER FROZEN STORAGE AND CONVERSION TO BACON¹

BY W. HAROLD WHITE²

Abstract

Determination of the peroxide oxygen and free fatty acid content of the fat of pork, stored under various conditions and subsequently converted to bacon, showed that temperature, method of wrapping, and stage in the conversion to bacon were the most important factors governing the oxidation and hydrolysis of the fat. Of the conditions studied, storage at temperatures of -18° to -23° C. with an aluminium foil wrapping, followed by thawing in brine or pickle were the most effective in retarding rancidity. The greatest increase in the peroxide oxygen content of the fat occurred during cure, whereas that of free fatty acid increased at a relatively uniform rate throughout the various conversion steps. Smoking had greater antioxidant effect on the fat than pale-drying. Since in all instances the content of free fatty acid was low, spoilage in pork or bacon fat is primarily due to oxidation.

Introduction

It is frequently necessary to store pork for the subsequent manufacture of Wiltshire bacon. The importance of this problem in Canadian agricultural economy has been outlined in previous publications (1, 3). In one of these (1) the effects of wrapping procedure, storage temperature, and method of thawing on colour and colour stability of the lean meat at various stages in the conversion of pork to bacon were given. The purpose of the present paper is to describe the effects of these same treatments on peroxide oxygen and free fatty acid formation in the fat.

Materials and Methods

The material and treatments employed have been described in detail previously (1). In brief, samples of pork back, wrapped in either a single or double layer of waxed paper or a single layer of aluminium foil and over-wrapped with a heavy Kraft paper, were stored in duplicate at temperatures of approximately -7° , -12° , -18° , -23° , and -29° C. (20° , 10° , 0° , -10° , and -20° F.) for 48 weeks. After cutting each back into two pieces, groups of 15 samples each were thawed in air, water, brine, or curing pickle. The samples were then cured in a commercial Wiltshire pickle at 4° C. (39° F.) for three and one-half days, matured for seven days at 3° F. (38° F.) and pale-dried or smoked for 7 hr. at 54° C. (130° F.). A small portion of the back fat was removed from each sample after each of the steps described above. In

¹ Manuscript received November 16, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Material selected from a thesis presented to the University of Western Ontario in partial fulfilment of the requirements for the degree of Master of Arts. Issued as paper No. 59 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 981.

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addition, since each back was divided into two at the termination of the storage period it was possible to compare the effect of the subsequent treatments on fresh surfaces and on those exposed during storage.

The peroxide oxygen and free fatty acid content of each of the samples of fat were determined by methods briefly described previously (3), and to be given in detail in a future publication. The selection of the two methods employed was based on the hypothesis that peroxide oxygen formation was indicative of oxidative changes whereas the presence of free fatty acid was primarily attributable to hydrolytic alterations in the fat.

Effect of Storage Conditions and Curing on Rancidity

The mean values of the peroxide oxygen and free fatty acid contents of the fat for each temperature, method of wrapping and of thawing, and stage of manufacture, averaged over all samples for the condition indicated, are given in Table I. The progressive increases in peroxide oxygen content noted for temperature changes from -18° to -12° , and from -12° to -7° C. all reached the level of statistical significance. The fat of pork stored at -7° C. and subsequently converted to bacon was definitely rancid; that held at -12° C. was well advanced in its induction period of rancidity. The values obtained for the lower temperatures were small, and approximate those of cured fresh pork. For periods comparable to that employed here a storage temperature of -18° C. is essential if spoilage of pork fat is to be avoided. Little apparent advantage is to be gained by the use of lower temperatures.

The free fatty acid content likewise significantly increased with increase in temperature. However, the concentrations present even at the higher temperatures were relatively low, and suggest that spoilage due to the formation of fatty acids is normally of little importance for pork fat.

The differences in peroxide oxygen content observed for each of the three types of wrappings were significant. Although a double wrap of waxed glassine paper was slightly more effective than a single wrapping in retarding oxidative rancidity, the impermeable aluminium foil covering was much superior to both these types. In contrast to peroxide oxygen, the free fatty acid content was greatest for those samples wrapped in aluminium foil, and least for those in the single wrap of waxed paper.

The differences in peroxide oxygen content observed between samples subjected to the various methods of thawing were not significant when considered for the experiment as a whole. However, thawing in brine or pickle usually yielded a product with slightly less peroxide oxygen than thawing in air or water. The explanation of this behaviour of the peroxide oxygen formation is not apparent. It may be that the lower solubility of oxygen in brine or pickle, as compared to water, or air itself, exercised a retarding effect. With regard to the content of free fatty acid, the results were similar. In this instance thawing in pickle gave a significantly lower mean value than in brine, which in turn was significantly lower than in air or water.

TABLE I

PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT OF PORK STORED AND CONVERTED TO BACON UNDER VARIOUS CONDITIONS

Factor	Peroxide oxygen ¹	Free fatty acid ²
	Mean	Mean
Temperature, °C.		
-7	31.8	2.09
-12	5.84	0.93
-18	2.27	0.64
-23	1.22	0.50
-29	1.33	0.62
Necessary difference	1.77	0.05
Method of wrapping		
Single wrap, waxed paper	11.1	0.78
Double wrap, waxed paper	9.47	0.82
Single wrap, aluminium foil	4.95	1.27
Necessary difference	1.37	0.04
Stage of manufacture		
After thawing	7.21	0.88
After cure	9.13	0.92
After maturation	9.16	1.06
Necessary difference	1.37	0.04
Method of thawing		
In air	8.73	1.05
In water	9.32	1.01
In brine	7.40	0.91
In pickle	8.54	0.85
Necessary difference	—	0.05

¹ Mean content as ml. 0.002N sodium thiosulphate over all conditions studied other than that indicated.

² Mean content as per cent oleic acid over all conditions studied other than that indicated.

Although the mean values of peroxide oxygen after maturation and after cure did not differ significantly, both were significantly greater than that obtained after thawing. Oxidative changes in the fat were therefore most extensive during cure. This may be due to the pro-oxidant effect of nitrite present in the curing pickle, or to the activation of the enzyme lipoxidase (4) by sodium chloride. Although significant increases in free fatty acid occurred between all three stages studied, the greatest change was during the maturation period.

The relative importance of the factors studied, in effecting changes in the peroxide oxygen and free fatty acid content, was assessed by means of an analysis of variance. Differences in the peroxide oxygen content, attributable to variations in temperature, wrapping, and stage of conversion, all

reached the level of statistical significance (Table II), whereas the method of thawing had little effect. Of the factors studied here, storage temperature and method of wrapping were of greatest importance. Similar general conclusions were reached with respect to the free fatty acid content (Table III). However, in this instance the method of thawing also had a significant effect.

Of the differential quantities, those relating temperature with the other three factors were usually of greatest influence. These have been graphically illustrated for both peroxide oxygen and free fatty acid in Figure I. The

TABLE II

ANALYSIS OF VARIANCE FOR THE PEROXIDE OXYGEN CONTENT OF
THE FAT OF PORK STORED AND CONVERTED TO BACON
UNDER VARIOUS CONDITIONS

Variance attributable to:	D.f.	Mean square
Temperature	4	6251 ***
Wrapping	2	606 ***
Thawing	3	28.8
Stage of conversion	2	75.2**
Temperature \times wrapping	8	448 ***
Temperature \times thawing	12	26.8*
Temperature \times stage	8	35.7*
Wrapping \times thawing	6	43.9**
Wrapping \times stage	4	28.3
Thawing \times stage	6	42.3*
Error	124	14.4

* Indicates 5% level of significance.

** Indicates 1% level of significance.

*** Indicates 0.1% level of significance.

TABLE III

ANALYSIS OF VARIANCE FOR THE FREE FATTY ACID CONTENT OF
THE FAT OF PORK STORED AND CONVERTED TO BACON
UNDER VARIOUS CONDITIONS

Variance attributable to:	D.f.	Mean square
Temperature	4	15.4 ***
Wrapping	2	4.42 ***
Thawing	3	0.396***
Stage of conversion	2	0.543***
Temperature \times wrapping	8	0.253***
Temperature \times thawing	12	0.221***
Temperature \times stage	8	0.031*
Wrapping \times thawing	6	0.032*
Wrapping \times stage	4	0.015
Thawing \times stage	6	0.005
Error	124	0.013

* Indicates 5% level of significance.

** Indicates 0.1% level of significance.

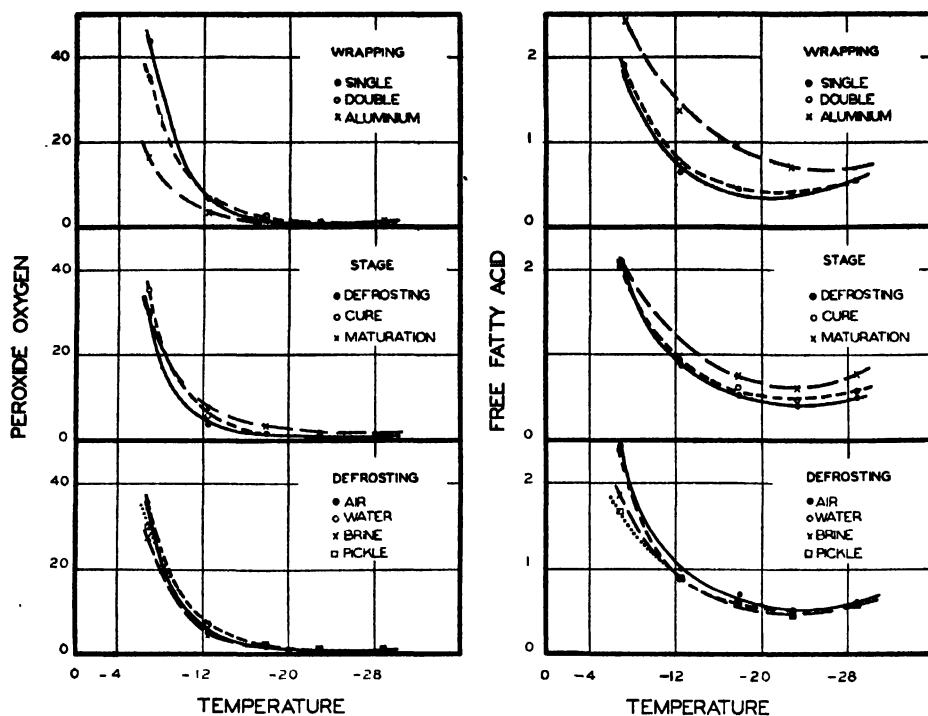


FIG. 1. *The differential effect between temperature and method of wrapping, thawing, and stage of conversion of pork to bacon on peroxide oxygen and free fatty acid formation in pork and bacon fat.*

curves show that the variations in the method of wrapping, thawing, etc. exerted their maximum effect on material stored at the higher temperatures, and that their influence decreased progressively with decrease in temperature. Since the fat of samples stored at -7°C . was well advanced in its induction period of rancidity and therefore especially susceptible to subsequent differences in treatment, these samples contributed much of the variance responsible for the significant interrelations.

The effect of position of sampling (internal or external, as previously described) was determined by the computation of t tests of significance between the two positions for each sample at all three stages. As would be expected the external samples in each instance contained significantly more peroxide oxygen than those from the internal position (Table IV). In addition the magnitude of the difference increased progressively with stage.

Position of sampling exerted an opposite effect on the free fatty acid content. For each stage the samples from the internal position contained significantly greater concentrations of free fatty acid than those obtained externally. This would suggest that oxidative changes contribute little to free fatty acid formation. However, the actual magnitude of the differences observed in free fatty acid content were small, and of little practical significance.

TABLE IV

t TESTS OF SIGNIFICANCE ON EFFECT OF POSITION FOR THE PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT OF PORK AT VARIOUS STAGES IN CONVERSION TO BACON

Property	Stage of conversion	Position with greater concentration	Mean difference	<i>t</i>
Peroxide oxygen ¹	After defrosting	External	4.76	2.65*
	After cure	External	4.72	2.87**
	After maturation	External	6.55	4.15**
Free fatty acid ²	After drefosting	Internal	0.12	2.35*
	After cure	Internal	0.26	5.95**
	After maturation	Internal	0.06	1.07

¹ As ml. 0.002*N* sodium thiosulphate.

² As per cent oleic acid.

* Indicates 5% level of significance.

** Indicates 1% level of significance.

Comparative Effect of Smoking and Pale-drying on Rancidity

When the free fatty acid and peroxide oxygen contents of the fat after smoking or pale-drying were compared by means of *t* tests with the corresponding values for after maturation, it was observed that in both instances significant increases had occurred (Table V). A comparison of the values for pale-dried samples alone with those for the corresponding samples after maturation showed that the peroxide oxygen and free fatty acid content had both increased significantly during pale-drying. A similar comparison for smoked samples showed that, although both the peroxide oxygen and free fatty acid content

TABLE V

t TESTS OF SIGNIFICANCE ON THE EFFECT OF SMOKING AND PALE-DRYING OF BACON ON THE PEROXIDE OXYGEN AND FREE FATTY ACID CONTENT OF THE FAT

Treatments compared	Treatment giving greater content	Mean difference	<i>t</i>
Peroxide oxygen content ¹ at:			
After smoking or pale-drying with after maturation	After pale-drying or smoking	3.15	4.39**
After pale-drying with after maturation	After pale-drying	4.82	4.07**
After smoking with after maturation	After smoking	1.48	1.90
Free fatty acid content ² at:			
After smoking or pale-drying with after maturation	After pale-drying or smoking	0.28	9.43**
After pale-drying with after maturation	After pale-drying	0.23	6.55**
After smoking with after maturation	After smoking	0.34	7.15**

¹ As ml. 0.002*N* sodium thiosulphate.

² As per cent oleic acid.

** Indicates 1% level of significance.

had increased, only that for free fatty acid reached the level of statistical significance. The peroxide oxygen content of the pale-dried samples was slightly greater than that of the smoked, but not significantly so. Smoked and pale-dried samples possessed approximately the same concentrations of free fatty acid.

Discussion and Conclusion

Although the reason for the variations in peroxide oxygen content as affected by the wrapping methods studied is unknown, it may be due either to the direct effect of greater exclusion of oxygen by the aluminium foil, or, indirectly, to the higher relative humidity within this form of wrapping. The presence of a high relative humidity may be associated with a retardation of surface desiccation, which causes a reduction in the effective area exposed to the air, and consequently of the rate of diffusion of oxygen into the fat. If the enhanced protection obtained by the aluminium foil were due merely to the exclusion of oxygen it might reasonably be expected to be independent of temperature. However, temperature had a significant effect on oxidation in the fat. Moreover, the quantity of oxygen required to cause rancidity in pork fat is small, and it may be reasonably assumed that more than sufficient would be present within any of the wrappings employed here. Consequently, it is considered that the development of rancidity in pork fat, as indicated by peroxide oxygen formation, at temperatures used in frozen storage, is retarded by high relative humidities. A similar observation was noted previously for frozen poultry (2).

The observed effect of wrapping on free fatty acid formation may be due to the progressive increase in relative humidity from the single waxed paper to the aluminium foil wrap, and its consequent effect on the growth of micro-organisms capable of effecting lipolysis of the fat. If so, the effect of wrapping should be greatest at the higher storage temperatures, which are most favourable for the growth of micro-organisms. Data given in Table VI for the mean values of free fatty acid for each wrapping at each of the temperatures studied shows this to be essentially true.

TABLE VI

MEAN VALUES OF THE FREE FATTY ACID CONTENT OF PORK FAT AFTER THAWING, FOR VARIOUS STORAGE CONDITIONS STUDIED

Temperature, °C.	Wrapping procedure		
	Single waxed paper	Double waxed paper	Aluminium foil
- 7	1.79†	1.94	2.53
-12	0.61	0.72	1.33
-18	0.42	0.33	0.86
-23	0.30	0.31	0.58
-27	0.46	0.45	0.58

† All values of free fatty acid as per cent oleic acid.

Since the peroxide oxygen content increased significantly after pale-drying but not after smoking, the smoke must have contained antioxidants. This is of interest since the possibility existed that the antioxidant effect of smoking might be due primarily to the inactivation of the oxidizing enzyme, lipoxidase, by heat. Although this investigation does not specifically show the effect of heat, since no tests were made on unheated bacon, the results indicate the antioxidant action of the smoking process is due at least in part to factors other than temperature.

From the results presented here it may be concluded that pork should be stored at temperatures of -18° to -23° C. for a period comparable to that employed here. Considerable protection from atmospheric oxidation is to be obtained by the use of moisture-impervious wrapping materials, such as aluminium foil. Although a double wrap of waxed paper was slightly better than a single wrap, it is doubtful whether it gave sufficient added protection to merit the extra cost. If frozen stored pork is to be subsequently converted to bacon, it should be thawed in a curing pickle or brine solution rather than in air or water. Since the over all changes in the free fatty acid content were low, deterioration in pork or bacon fat as the result of hydrolytic changes would appear to be of little importance. Decomposition due to oxidation is therefore primarily responsible for spoilage.

Acknowledgments

The author wishes to thank Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice, and to acknowledge the technical assistance of Mr. E. G. Blake, Laboratory Assistant.

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CANADIAN WILTSHIRE BACON

XVIII. EFFECT OF TEMPERATURE AND BACTERIAL GROWTH ON NITRITE CONTENT¹

BY W. HAROLD WHITE² AND N. E. GIBBONS³

Abstract

Samples of bacon, with bacterial populations adjusted to two levels, were held at temperatures of 4°, 21°, 38°, and 55° C. for 20, 40, 80, and 160 hr. Both the total number of organisms and the number capable of reducing nitrate to nitrite were significantly correlated with the nitrite content. Furthermore, the samples adjusted to the high bacterial level usually contained more nitrite after treatment than those from the same hog but containing fewer bacteria. The increase in nitrite, observed here and previously, at temperatures below 55° C., is attributable primarily to bacterial growth and not to enzymes or other constituents of the bacon.

Introduction

In a previous investigation (6), it was found that, as in the smoking or pale-drying processes, the nitrite content of small cuts of bacon increased upon heating at temperatures below 55° C. Since the amount of nitrite affects both the colour and colour stability of bacon (7, 8), further studies were undertaken to determine the factors responsible for the increase in nitrite content.

It was suggested in a previous paper (6) that the growth of bacteria might be responsible for the increases in nitrite. In support of this it was observed that, upon heating, the nitrite content reached a maximum at 40° C., a temperature at which rapid bacterial growth might be expected. Furthermore, comparable increases did not occur in whole sides (1), possibly because of the greater opportunity for contamination and the greater exposure of the substrate to bacterial action in the small pieces. However, it was possible that certain enzymes or other constituents or properties of the meat were responsible for the reduction of nitrate. The present investigation was undertaken to determine the extent to which bacteria were responsible for the observed changes, and at the same time to obtain indirect evidence of the importance of other agencies.

Material and Methods

The material consisted of two rib-in export backs removed from each of three hogs that had been cured together in the same tank and treated as nearly alike as possible within the limitations of commercial plant practice.

After cure the six backs were incubated for two days at 20° C. in order to increase the bacterial population, while maintaining comparable nitrite levels.

¹ Manuscript received December 4, 1940.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 60 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 982.

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In order to ensure the existence of two bacterial levels, the ribs of three sides, selected so that each hog was represented, were scraped, and the exposed lean surface scrubbed and washed with sterile 4% sodium chloride solution. The lean portion of each of the three backs was ground and mixed by several passages through a sterile food chopper in a room at 4° C. The lean from each of the remaining backs, without washing or scrubbing, was mixed with the surface scrapings and ground to give three samples of higher bacterial content.

Fifty-gram subsamples of the ground material from each of the six backs were placed in sterile, sealed, glass containers, and held in air ovens at temperatures of 4°, 21°, 38°, and 55° C. for 20, 40, 80, and 160 hr. In addition to these 96 samples, subsamples of each ground back were taken at the beginning, middle, and end of the weighing procedure in order to obtain representative values for the initial bacterial and nitrite content. The selection of temperatures was based on the following considerations: 4° C. represented the normal temperature for curing and maturation; 21° C. was the temperature previously used for bacteriological studies on bacon (2); at 38° C., maximum nitrite production was noted previously; a temperature of 55° C. had been previously found to have little effect on the nitrite content (6). At the termination of the desired treatment the samples were brought rapidly to room temperature (approximately 20° C.), weighed, thoroughly mixed, and sampled for bacteriological study. They were then frozen immediately and stored at -40° C. until analysed chemically.

The nitrite content of the meat after the various treatments was determined in duplicate by methods previously described (5). For bacteriological examination duplicate 5-gm. samples of the ground meat (10 gm. for the initial samples) were shaken 200 times in bottles containing 4% sodium chloride solution and coarse silica sand. All subsequent dilutions were made with 4% sodium chloride solution. Agar containing 4% sodium chloride was used throughout (2). Plates were incubated at 20° C. for six days. However, at the final sampling duplicate sets of plates for the meat samples held at 38° C. and 55° C. were also incubated at the corresponding temperatures for three days.

To determine the proportion of the total bacterial population capable of reducing nitrate, all of the colonies from a plate or section of a plate were inoculated directly into beef extract broth containing 4% sodium chloride and 0.1% potassium nitrate. After four-days' incubation at 20° C. these tubes were tested for the presence of nitrite in the customary manner (3). If no nitrite was present, a test was made for nitrate by reduction with zinc dust (3) to determine whether reduction had not occurred, or whether it had been carried beyond the nitrite stage. Of some 3700 cultures examined only four reduced all of the nitrate beyond the nitrite stage. Although 100 colonies were usually selected for each test, this was at times impossible. However, unless 25 colonies were available the results are not presented. The proportion of nitrate-reducing organisms was estimated from tubes showing definite growth.

Results

The results for the effect of temperature on the nitrite content of Wiltshire bacon, presented as the means of the six sides regardless of bacterial level (Table I), show close agreement with those obtained in the previous investigation (6). However, the actual magnitude of the present changes was somewhat greater than of those noted previously. This is presumably due to a greater average bacterial population and to the relatively greater area of exposed surface.

TABLE I

MEAN NITRITE CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS
(AS P.P.M. ON DRY MATTER BASIS)

Period of heat treatment, hr.	Temperature of heat treatment, °C.			
	4	21	38	55
0	51	51	51	51
20	48	359	3249	23
40	51	1403	2966	27
80	49	2481	3079	149
160	110	4082	1549	304

Except in the 160-hr. treatment, the nitrite content increased with increase of temperature to a maximum at 38° C., and then decreased to approximately the original level, at 55° C. However, as the length of the heating period increased, the differences in content between 21° and 38° C. decreased, until at 160 hr. the greatest value was obtained at 21° C.

The total number of organisms showed the same behaviour as the nitrite content (Table II). The most rapid bacterial development occurred at 38° C., reaching a maximum between 80 and 160 hr. At 4° and 21° C., the number of bacteria continued to increase, attaining the greatest observed value after 160 hr.

TABLE II

MEAN TOTAL BACTERIAL CONTENT OF BACON AFTER VARIOUS HEAT TREATMENTS
(AS LOGARITHM OF NUMBER OF ORGANISMS PER GM. OF MEAT)

Period of heat treatment, hr.	Temperature of heat treatment, °C.			
	4	21	38	55
0	5.74	5.74	5.74	5.74
20	5.90	7.46	7.70	1.30
40	6.02	7.72	7.76	5.19
80	6.16	7.93	9.08	4.38
160	8.07	8.93	8.60	3.42
160	—	—	8.75 ¹	4.37 ²

¹ Plates incubated at 38° C.

² Plates incubated at 55° C.

TABLE III

NITRITE CONTENT, AS P.P.M., AND BACTERIAL CONTENT, AS LOGARITHM OF NUMBER OF ORGANISMS PER GM., OF BACON HEATED AT 55° C. FOR VARIOUS PERIODS

Time of treatment, hr.	Sample											
	1		2		3		4		5		6	
	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria	Nitrite	Bacteria
20	27	<1 3	30	<1 3	25	<1 3	19	<1 3	24	<1 3	15	<1 3
40	20	6 41	24	6 91	12	<1 3	38	7 69	57	7 52	9	<1 3
80	778	8 35	34	>7 0	65	>7 0	6	<1 3	3	<1 3	6	<1 3
160	6	<3 3	6	<3 3	5	<3 3	393	<1 3 ¹	9	<1 3	1406	8 00

¹ Count at 55° C. = 6.23.

There was considerable variation in the bacterial and nitrite content of the samples held at 55° C. In general the number of organisms decreased rapidly. However, at 40 hr., four samples, 80 hr., three samples, and 160 hr., one sample had high counts (Table III). One sample, held for 160 hr., had a low count, as shown on plates incubated at 20° C, but a high count on plates incubated at 55° C. This was the only instance in which plates incubated at either 38° or 55° C. had an appreciably different count from those incubated at 20° C. The plates with high counts were practically pure cultures of a spore-bearing, nitrate-reducing rod. This organism, which was apparently scattered sparingly through the original samples and of a type rarely found in bacon, accounted for the high nitrite values and high bacterial counts observed after 80 and 160 hr. Actually the only samples with high nitrite contents were those having high bacterial counts.

The percentages of the total number of organisms capable of reducing nitrate to nitrite are shown in Table IV. Not only were the majority of the organisms able to reduce nitrate to nitrite, but the number of reducing organ-

TABLE IV

PERCENTAGE OF TOTAL NUMBER OF ORGANISMS CAPABLE OF REDUCING NITRATE TO NITRITE IN BACON AFTER VARIOUS HEAT TREATMENTS

Period of heat treatment, hr.	Temperature of treatment, °C.			
	4	21	38	55
0	91	91	91	91
20	86	91	74	—
40	90	96	98	100
80	84	79	47	—
160	90	67	85 ¹	—

¹ 83% when incubated at 38° C.

isms was highly correlated with the total number ($r = 0.95$, which exceeds the 1% level of significance for the 66 degrees of freedom available). Although the total count was obtained by accepted aerobic procedures, conditions existing in the determination of nitrate reduction and in the meat ranged from aerobic to essentially anaerobic. It has been stated (4) that nitrate is reduced by pure cultures of organisms under aerobic or anaerobic conditions and it would appear that reduction takes place under both conditions in meat.

In Fig. 1 the mean values of the nitrite content and total count for each of the two bacterial levels are plotted against temperature for each of the periods studied. The close similarity in the nature of the curves for the nitrite and bacterial content is evident. Moreover, the samples adjusted to the high bacterial level contained more nitrite in most instances than similar samples containing fewer bacteria, although the difference between the two levels in the original samples was not large (factor of 4 to 6).

The magnitude of the apparent inter-relation between the nitrite content and the bacterial count was determined by means of simple coefficients of correlation. Such a computation for nitrite and total bacterial content indicated that the two quantities were significantly correlated (Table V).

TABLE V

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE NITRITE CONTENT AND TOTAL NUMBER OF ORGANISMS IN BACON AFTER VARIOUS HEAT TREATMENTS

QUANTITIES CORRELATED	D.f.	r
Logarithm of nitrite content with logarithm of total number of organisms for the following conditions:		
All treatments	94	0.72**
All treatments less 160 hr.	70	0.76**
All treatments less 80 and 160 hr.	46	0.69**
All treatments less 55° C.	70	0.69**
All treatments less 4° and 55° C.	46	0.44**
All treatments less 38° and 55° C.	46	0.68**
All treatments less 160 hr. at 55° C.	52	0.84**
All treatments less 160 hr. at 4° and 55° C.	34	0.47**
All treatments less 160 hr. at 38° and 55° C.	34	0.80**
All treatments less 80 and 160 hr. at 55° C.	34	0.85**
All treatments less 80 and 160 hr. at 4° and 55° C.	22	0.83**
All treatments less 80 and 160 hr. at 38° and 55° C.	22	0.80**
All treatments less { 4° and 55° at 20, 40, and 80 hr.	40	0.71**
All treatments less { 4°, 38°, and 55° C. at 160 hr.		
All treatments less { 4° and 55° at 20 and 40 hr.	34	0.52**
All treatments less { 4°, 38°, and 55° at 80 and 160 hr.		
Logarithm of increase in nitrite content with logarithm of increase in total number of organisms for the following conditions:		
All treatments less { 20, 40, and 80 hr. at 55° C.	43	0.61**
All treatments less { 160 hr. at 38° and 55° C.		
All treatments less { 4° and 55° C. at 20, 40, and 80 hr.	40	0.54**
All treatments less { 4°, 38°, and 55° C. at 160 hr.		
All treatments less { 4° and 55° C. at 20 and 40 hr.	34	0.52**
All treatments less { 4°, 38°, and 55° C. at 80 and 160 hr.		

** Indicates 1% level of significance.

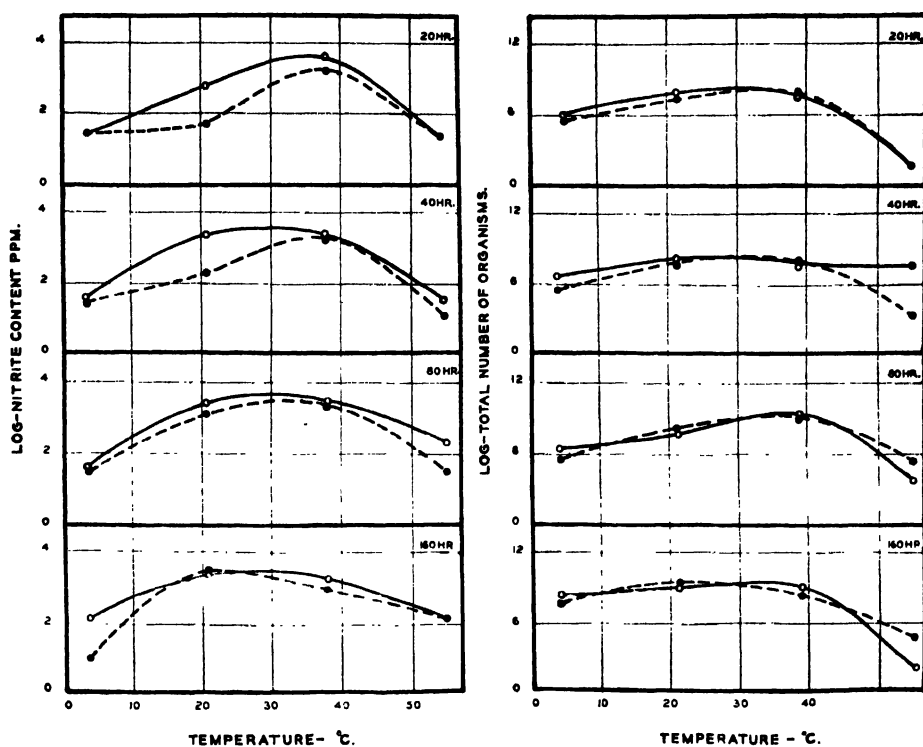


FIG. 1. Effect of time and temperature on the nitrite content and total bacterial count of Wiltshire bacon. — Samples adjusted to high bacterial count. - - - - Samples adjusted to low bacterial count.

However, only about half of the observed variance was accounted for by this correlation. Since the nitrite present may react with the meat proteins and pigments, be oxidized to nitrate, or reduced to other compounds of nitrogen, it is apparent that the value obtained experimentally is dependent on these factors as well as bacterial reduction from nitrate. Moreover, it may reasonably be assumed that both temperature and time have a differential effect on the reactions causing a decrease in the nitrite content.

Evidence of the differential effects due to time and temperature are shown by the results in Table III, and by the fact that the greatest values for nitrite and bacterial content occurred at 38° C. for the shorter periods of treatments but at 21° C. for the 160-hr. period. More definite proof was obtained by computing simple coefficients of correlation, in which the data obtained for one or more of the conditions was omitted (Table V). Although it is unnecessary to discuss these at length, it is of interest to note that the highest correlations were obtained when the data for heating at 55° C. for 80 and 160 hr. were excluded. This suggests that the greatest decomposition of nitrite occurred under these conditions. The magnitude of the correlation coefficient was such that 72% of the variance was accounted for. Since the combined sampling and experimental errors could account for most of the residual

variance, it is concluded that the formation of nitrite and bacterial growth in bacon are closely related. Statistically significant relations were also obtained between the logarithms of the arithmetic increases in nitrite and total bacterial content.

The results of calculations of correlation coefficients between the nitrite content and the number of nitrate-reducing organisms and between the increases in the logarithms of these quantities confirm in general the above described observations (Table VI). The smaller magnitude of these coefficients is presumably due to the greater experimental error introduced in the determination of those organisms capable of reducing nitrate.

TABLE VI

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE NITRITE CONTENT AND NUMBER OF NITRATE-REDUCING ORGANISMS IN BACON AFTER VARIOUS HEAT TREATMENTS

Quantities correlated	D.f.	
Logarithm of nitrite content with logarithm of number of nitrate-reducing organisms for the following conditions:		
All treatments ¹ less 160 hr.	47	0.77**
All treatments ¹ less 80 and 160 hr.	28	0.68**
All treatments ¹ less 55° C.	60	0.67**
All treatments ¹ less 4° and 55° C.	37	0.41*
All treatments ¹ less 38° and 55° C.	41	0.67**
All treatments ¹ less 160 hr. at 55° C.	42	0.83**
All treatments ¹ less 160 hr. at 4° and 55° C.	27	0.49**
All treatments ¹ less 160 hr. at 38° and 55° C.	29	0.79**
All treatments ¹ less 80 and 160 hr. at 55° C.	24	0.81**
All treatments ¹ less 80 and 160 hr. at 4° and 55° C.	13	0.68**
All treatments ¹ less 80 and 160 hr. at 38° and 55° C.	17	0.79**
All treatments ¹ less { 4° and 55° C. at 20, 40 and 80 hr.	31	0.35*
All treatments ¹ less { 4°, 38°, and 55° C. at 160 hr.		
All treatments ¹ less { 4° and 55° C. at 20 and 40 hr.	25	0.48*
All treatments ¹ less { 4°, 38°, and 55° C. at 80 and 160 hr.		
Samples in which nitrite content increased	43	0.79**
Increase in logarithm of nitrite content with increase in logarithm of nitrate-reducing organisms for the following conditions:		
All treatments less 55° C.	41	0.40**
All treatments less 160 hr. at 55° C.	26	0.45*
All treatments less 160 hr. at 38° and 55° C.	13	0.45
All treatments less 80 and 160 hr. at 55° C.	12	0.18
All treatments less 80 and 160 hr. at 38° and 55° C.	5	0.38

¹ Data for periods at 55° C. incomplete.

* Indicates 5% level of significance.

** Indicates 1% level of significance.

Although it has been shown conclusively that the contents of nitrite and bacteria were closely correlated, the possibility existed that conditions favouring the growth of bacteria might also be most suitable for nitrite production by some other agency, e.g., a tissue enzyme of bacon. However, since samples treated to give a higher bacterial content usually contained greater quantities

of nitrite than those from the same hog adjusted to a lower bacterial level, and since at 55° C. the amount of nitrite decreased except where growth of the more thermophilic organisms occurred, it is considered that this possibility is excluded. The increase in nitrite at temperatures below 55° C. observed in this and a previous investigation (6) must then be due primarily to reduction from nitrate by the action of bacteria.

Acknowledgment

The authors wish to acknowledge the technical assistance of Messrs. E. G. Blake and A. J. McLeod, Laboratory Assistants, National Research Laboratories.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

APRIL, 1941

NUMBER 4

STUDIES OF WATERFOWL IN BRITISH COLUMBIA

GREATER SCAUP DUCK, LESSER SCAUP DUCK¹

By J. A. MUNRO²

Abstract

Nyroca marila is an abundant migrant through British Columbia and large numbers winter in the coast region. The sex ratio in winter flocks is predominantly male. *Chara* was the chief food eaten by 57 specimens from Okanagan Lake, miscellaneous vegetable matter was second, and molluscs third in importance. Food items, listed in order of importance, on coast streams and lakes were: vegetable matter, molluscs, salmon eggs, salmon flesh, and, on salt water: gastropods, sea lettuce (*Ulva* sp.), crustaceans, and herring eggs. *N. affinis* nests commonly in parts of the dry interior and elsewhere in the province is a migrant and scarce winter visitant. Sex ratio is predominantly male. Courtship continues through April and May; laying commences in June and late clutches are found in August. Females defend their young vigorously and a habit of combining broods has a probable survival value. Males raft on certain lakes in July and go into eclipse as flight feathers are shed. These populations include yearling and post-breeding females and later, adolescents. The former moult at this time. Adults migrate early and those remaining are largely young of the year. Amphipods are the chief food of all age groups on the nesting ground; aquatic insects and seeds of aquatic plants are also important. Both species of scaup ducks are economically important as food and for sport in the interior but less so on the coast where, because of a different diet, their flesh is less palatable. It was not determined whether the consumption of salmon eggs and herring eggs is of economic significance. Elsewhere than on the coast scaup ducks are related to other interests only to the limited extent to which they are food competitors of trout and other commercially valuable fishes.

Introduction

The present paper is submitted as a contribution to the life histories of the scaup ducks as observed in British Columbia. In the case of the Greater Scaup Duck, *Nyroca marila* (Linn.), known only as a migrant and winter visitant, the data concern chiefly its winter range, numerical status, and food habits. Concerning the Lesser Scaup Duck, *Nyroca affinis* (Eyton), which nests commonly in parts of the province, more detailed information, particularly about behaviour of summer populations, is presented.

A study of the migratory movements of the scaup ducks is complicated by the fact that the two species resemble each other closely and much observational data cannot be accepted. Although they may be separated readily enough in the hand, it is usually more difficult and sometimes impossible to

¹ Manuscript received December 9, 1940. Contribution from the National Parks Bureau, Department of Mines and Resources, Ottawa, Canada.

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do so in the field. The smaller size of *N. affinis*, the smaller amount of white on its wing and the specific characteristic of greenish or purplish head reflections in adult males are relative characters apparent only under favourable conditions. Nevertheless from many observations in the field and from the more satisfactory evidence afforded by specimens examined closely, it seems clear that the winter population in British Columbia is predominantly the species, *N. marila*, and the nesting population is exclusively *N. affinis*.

Field observations and records of stomach analyses covering the period 1911 to 1940 form the basis of this paper. As in earlier contributions of a similar nature, the food studies were largely a joint undertaking by Dr. W. A. Clemens, then Director of the Pacific Biological Station, and the author. The methods of laboratory examination described by Munro and Clemens (13) were followed.

Greater Scaup Duck

DISTRIBUTION ON THE PACIFIC COAST

There are no records of the Greater Scaup Duck nesting in British Columbia nor in the United States south of this province. Except for the probable occurrence in summer of non-breeding individuals, its status is that of a winter resident. The breeding grounds lie well to the north and east of British Columbia. In winter it is abundant on the coast of southeastern Alaska, British Columbia, and Washington. It is not recorded by Jewett and Gabrielson (6) from the Portland area, Oregon, and once only from Netarts Bay (17). Further investigation in Oregon may prove the species to be more plentiful than the available records indicate since it is a common midwinter visitant to the coast of northern California. According to James Moffitt (personal letter, January 30, 1940), as many as 6000 have been observed at Tomales Bay (January 20, 1929) and 4000 on Humboldt Bay (January 19, 1932).

Spring Migration

In parts of the interior of British Columbia, the Greater Scaup Duck is a regular spring migrant, arriving somewhat earlier than the Lesser Scaup Duck and remaining on some of the larger lakes where food is plentiful for a month or six weeks. On the open water of the Thompson River, small flocks have been reported in February (73 at Kamloops, February 10, 1940). In the Okanagan first arrivals are usually observed in March, for example, 32 at Okanagan Landing, March 28, 1932. By the latter part of April, the migration of *N. marila* is nearly over whereas that of the *N. affinis* is at its height. Thus on a series of small ponds near Kamloops (April 25, 1939) only 35 in a total of 500 scaup ducks were identified as *N. marila*.

The greater part of the winter population, however, goes slowly up the coast, their movements governed largely by the movements of Pacific herring which at this time (late February to early April) are spawning. During the height of the spawning runs the largest number of greater scaups are present and the greatest concentrations take place. The following estimates indicate the usual size of these flocks: 2000 at Departure Bay, March 3, 1928; at

Nanoose Bay, 4500 on March 11, 1928; 4000 on March 10, 1931; 3000 on March 26, 1936; 8000 on March 21, 1940; 1500 at Qualicum Beach, March 15, 1939. Late dates for spring migrants are: Victoria, May 14, 1904; Clayoquot, May 22, 1931; Masset Inlet, Queen Charlotte Islands, May 26, 1920. The last refers to a flock of 30 that were chiefly yearling males.

Autumn Migration

The difficulty of distinguishing between the species of scaup ducks has been pointed out. This is particularly the case in the autumn when there is the maximum association of the two species and the birds are so wary that close observation usually is impossible. At this time also there is difficulty in sight sex determination as some adult males are in eclipse and young males show white cheek patches similar to those worn by the adult female.

In the Okanagan Valley the first fall migrants appear in early October (specimens taken at Swan Lake, October 6, 1933, and October 4, 1934).

The fall population in the Okanagan reaches its maximum usually in early November and the majority disappear with the first freeze up, which normally occurs toward the end of that month. Subsequently, small flocks remain for a time on such of the larger lakes as have remained open but it has been unusual for the past 15 years for any to be present after late December.

Along the southern part of the coast region early arrivals are reported in September, for example at Comox, September 11, 1922, 25 were counted (Canadian National Museum record). The first large flocks usually are seen in early October, for example, 200 at Oak Bay, October 13, 1912. The migration continues through October and reaches its peak in November, after which the numbers decline. There remains a winter population (December-February) fluctuating in numbers and, in part, moving from salt water to fresh water feeding grounds.

Winter Populations

In earlier years several hundred greater scaups usually wintered on Okanagan Lake near its northern end and smaller numbers near the south end of this lake. In both places are shallow feeding grounds that completely freeze over only in exceptionally cold weather. Thus in January, 1918, a total of 150 and, in February, a total of 50 was counted whereas in March the number increased, by the addition of migrants, to 400. In 1919, a raft of approximately 200, of which about 70% were males, remained in the vicinity for the same period. They were last seen in such numbers in February, 1921, and since that time the records refer to flocks numbering up to 50 observed usually no later than December, for example, 25 on December 31, 1930; 2 on December 14, 1931; 50 on November 14, 1932; 12 on December 30, 1932; 36 on December 23, 1938.

In the mild winter of 1939-40, a flock of 25 wintered on the Thompson River near Kamloops but, in general, winter populations in British Columbia are now chiefly restricted to the coast region, where flocks up to 500, or more,

frequent suitable waters. In some localities, particularly the several bird sanctuaries comprising sheltered tidal waters on Vancouver Island, they become quite fearless of man and may be studied at close range, thus simplifying the matter of identification. Normal-sized flocks are indicated by the following counts: 149 at Nanaimo Harbour, January 7, 1939; 150 at Departure Bay, January 26, 1935; 400 at Ross Bay, February 16, 1940; 300 at Shoal Bay, February 16, 1940.

Sex Ratio of Greater Scaup Duck

In all flocks of Greater Scaup Ducks examined in winter, an excess of males over females has been observed. In large flocks examined at a distance it has not usually been possible to make exact counts but estimates suggest a proportion of six or seven males to one female. Where winter flocks were examined at close range and precise determination made, it was observed that the sex ratio varied, for example: 61 ♂ and 7 ♀ at Esquimalt Lagoon, February 26, 1940; 60 ♂ and 40 ♀ at Departure Bay, March 20, 1940.

FOOD AND BEHAVIOUR OF GREATER SCAUP DUCK IN THE INTERIOR OF BRITISH COLUMBIA

When present on one of the large lakes in the interior of British Columbia a flock of Greater Scaup Ducks may form part of a raft of diving ducks that have gathered on a rich feeding ground, such as a bed of *Potamogeton* or *Chara*. In these associations of diving ducks, which nearly always include American Coot, *Fulica americana*, and Baldpate, *Mareca americana*, the scaups usually form a unit; less often they are scattered through the raft. The waterfowl making up these large flocks invariably are wary. They stay well out from shore and, if not disturbed, may remain, feeding and resting, in the same locality during the greater part of the day.

When associated in small flocks, the greater scaups usually are less nervous and often feed close to shore. At such times all the members of a small flock up to a dozen or so sometimes submerge almost simultaneously, a habit that may be used to advantage by hunters on shore.

After the hunting season, certain small flocks in the vicinity of settlements become quite tame. Thus at Okanagan Landing in the winter of 1920-21, a small number congregated about the wharf each day when the steamer, then in service on the lake, tied up. These birds in response to a whistled call would swim quickly towards the wharf and feed upon scraps thrown to them within a few yards of interested spectators.

The leaves of *Potamogeton* and branchlets and oospores of *Chara* are prominent items of diet. It is known that *Chara* may suddenly become scarce or disappear from places where it has been abundant and not become re-established for several years. Possibly this is a factor influencing the local distribution and abundance of the Greater Scaup Duck and other species of diving ducks in the interior.

Quite recently a changed environmental condition has been brought about in a part of Okanagan Lake through the rapid replacement of *Chara*, the most valuable food in the lake, by the less desirable water weed, *Elodea canadensis*. Okanagan is a deep lake with only limited areas of feeding grounds and over much of these areas *Chara* was dominant until 1937. It grew profusely and in places the broken residue piled up on the shore to a depth of 12 in. or more. About 1936, *Elodea* appeared in quantity; at present (1940) it is dominant over much of the feeding ground near Okanagan Landing and drifted shore accumulations of aquatic vegetation is composed almost exclusively of this plant.

FOOD SUMMARIES

In the section following is given a summary of the food eaten by the Greater Scaup Duck in the Okanagan District, British Columbia, as indicated by the examination of the contents of 79 stomachs from two localities, namely, Okanagan Lake and Swan Lake.

The Okanagan Lake material, collected in the winter chiefly from 1912 to 1915, is of some historic interest as it reflects a condition that has passed away. It has been mentioned that in the period stated and for some years later the Greater Scaup Duck wintered regularly on Okanagan Lake and that since that time it has practically disappeared as a member of the midwinter bird life. The ecological modification of the area has been referred to.

In the following summary the number of specimens examined is indicated by the figure following each of the months represented.

Okanagan Lake—November, 3; December, 9; January, 28; February, 26; March, 2.

Unidentified fishes. A trace of fish remains was a minor item in one and represented 2% of the contents of another stomach.

Crustaceans. The single occurrence of an amphipod represented 1% of the stomach contents; an isopod was a minor item in another specimen.

Caddis. In eight analyses caddis was found in four specimens, constituting less than 1%, and 2 to 12% of the food eaten, in the remainder.

Other insects. Fragments of Coleoptera bodies constituted 10% of the material in one, and less than 1% in three, stomachs. Chironomid larvae had been eaten by one specimen and represented 25% of the stomach contents. Corixids, Zygoptera, and Hymenoptera (ants) were each represented once as minor items.

Chara sp. Branchlets and oospores of *Chara* were found in all but five of the fifty-eight stomachs and were the chief or exclusive items in 43 specimens.

Sagittaria sp. Ground up fragments of *Sagittaria* tubers were the exclusive item in four, and represented 50 and 75% of the contents of two other stomachs.

Potamogeton sp. Stems, winter buds, and rootlets occurred in 10 stomachs, in six specimens representing 65 to 100% of the total contents; seeds of several species occurred in nine stomachs.

Miscellaneous vegetation. To this category are referred 23 records of plant fragments including *Zanichellia palustris*, *Rupia maritima*, and unidentified species. Material of this nature was the chief item in six specimens.

Miscellaneous seeds. Seeds of the following plants were represented the number of times indicated: *Scirpus americanus*, 7; *Polygonum* sp., 3; *Polygonum amphibium*, 2; *Cornus* sp., 3; *Najas flexilis*, 2; *Amaranthus* sp., 1; *Batrachium* sp., 1; *Hydrocotyle* sp., 1; *Triticum* sp., 1.

Gastropods. Snails, including *Limnaea palustris*, *Limnaea vahli*, *Planorbis* sp., and *Valvata virens*, represented respectively 14, 25, 42, and 98% of the contents of four and were present as minor items (8 to less than 1%) in 11 other stomachs.

Pelecypods. One specimen taken in March contained a whole specimen of *Anodonta* sp. measuring 45 mm. Other species represented were: *Pisidium additum* and *Sphaerium occidentale*, which occurred 15 times; they usually represented a small proportion of the contents but in one case constituted 46, and in another 53% of the total contents.

Summary.—*Chara* is the most important food both in frequency of occurrence and average percentage volume; a decided preference for this food is indicated. *Potamogeton* and other vegetable matter are second and mollusca third in total percentage volume; insects are of slight importance in the winter months.

Swan Lake, Okanagan,—November, 7; December, 4.

This is a shallow marshy lake three and one-half miles long which freezes over in winter. The specimens were taken here in the autumn of 1939, a year in which the usually abundant *Chara* was scarce.

Aquatic insects.

A specimen taken in December had eaten over 100 chironomid larvae (bloodworms); corixids and dragonfly nymphs each occurred twice.

Molluscs. Small specimens of *Planorbis* were present in one, and fragmentary mollusc shells in a second, stomach.

Seeds. In several specimens a few seeds of *Ceratophyllum demersum* were the only item of food; other species represented by seeds were: *Potamogeton pectinatus*, *Scirpus americanus*, *Zanichellia palustris*, and *Carex* sp.

Miscellaneous vegetation. Comminuted vegetable matter was identified in one case as *Potamogeton pectinatus*, in another as *Ceratophyllum demersum*.

Summary.—Seeds are the chief item in frequency of occurrence and average percentage volume. Miscellaneous vegetable matter is second and miscellaneous aquatic insects third in importance.

FOOD AND BEHAVIOUR OF GREATER SCAUP DUCK IN THE COAST REGION
OF BRITISH COLUMBIA

Salmon ova, which become available soon after the greater scaups have arrived on the southern British Columbia coast, are a seasonal food of some importance. Spring salmon, *Oncorhynchus tshawytscha*, begin to ascend the coast streams with the first fall freshets; then come coho salmon, *Oncorhynchus kisutch*, sockeye salmon, *Oncorhynchus nerka*, pink salmon, *Oncorhynchus gorbuscha*, and chum salmon, *Oncorhynchus keta*. The spawning season of the five species covers the period from October to December inclusive. At this time, on the part of the ducks and gulls that feed upon salmon eggs, there is great activity and constant movements from the ocean to the streams.

The Greater Scaup Ducks may visit a spawning stream at any time of the day and remain for only a short time or they may arrive shortly after dawn and remain until dusk. No evidence of night feeding has been obtained. All appear to spend the night on the sea or on lakes drained by the spawning streams. The number visiting the streams varies from week to week, more being present during the actual spawning periods, and for a few days afterward, than at other times. In a few days after a heavy spawning, drifting eggs are easily obtainable from the bottom of the stream. The eggs taken appear to be exclusively those that have not been covered with gravel during the spawning or that have been dislodged during subsequent spawnings by freshets or some other agency. No evidence that the Greater Scaup Duck extracts eggs out of the redds has been obtained.

The rotted flesh of dead salmon that have stranded along the spawning stream also is eaten at this season. The gullets of two greater scaups, shot on the Goldstream river in November, 1938, were filled with this semi-liquid material.

Perhaps the most important seasonal food is the ova of Pacific herring, *Clupea pallasii*, and when the spawning season is at its height in March and early April large concentrations of greater scaups and other diving ducks take place over the spawned areas and on adjacent waters. Usually the different species are more or less segregated, forming an association of flocks rather than of individuals, but after being flushed several times these units tend to break up and the different species to mix indiscriminately. For example, at Nanoose Bay (March 12, 1934) several thousand Greater Scaup Ducks in a single flock were close to, but not intermingled with, smaller flocks of White-winged Scoters, *Melanitta deglandi*, and Surf Scoters, *Melanitta perspicillata*. After these had been disturbed by a passing launch, no further segregation was observed during that day. Upon another occasion at the same place (March 26, 1936) approximately 3000 Greater Scaup Ducks were associated with a smaller number of White-winged Scoters and Surf Scoters but no segregation of species was apparent. In these large rafts of ducks the greater scaup is invariably the most wary of the species assembled and when approached by boat all will take wing almost simultaneously, several

minutes before ducks of other species show signs of alarm. The relative ease with which the greater scaups leave the water, their tendency to attain altitude immediately, the compact nature of the flock, and the mobility of the birds in the air is in marked contrast to the less agile movements of the other diving ducks as they rise.

Herring deposit their eggs on various kinds of algae or on eel-grass, *Zostera marina*, in relatively shallow areas close to shore. Those that do not adhere to these growths sink to the bottom and accumulate amongst the vegetation or in depressions on the sea bottom. These deposits of unattached eggs are considered to be the chief source of supply for the greater scaup and other diving ducks.

Only one specimen of greater scaup shot on the spawning grounds was available for examination but it has been observed that their method of obtaining this food is similar to that of White-winged Scoters, Surf Scoters, American Golden-eye, *Glaucionetta clangula americana*, Buffle-head, *Charitonetta albeola*, and Old Squaw, *Clangula hyemalis*. All obtain the eggs by diving; none have been observed on the spawning grounds when the eggs are exposed at low tide. The stomachs of these ducks contained usually a mixture of herring eggs and sand (indicating that the material had been scooped from the sea bottom) and infrequently a mixture of herring eggs and vegetation.

Salmon eggs and herring eggs are seasonal foods only; the staple item of diet from salt water consists of various small molluscs that are obtained by diving close to shore. Crustaceans obtained in similar situations are a food of less importance. Sea lettuce, *Ulva* sp., is eaten extensively and small flocks of greater scaups frequently have been watched as they dived for this food. Thus at Departure Bay (February 18, 1934) four adult males were observed from a concealed position almost directly above them. From this vantage point all objects on the sea bottom and each movement of the active ducks was clearly visible. Glistening air bubbles adhered to their white flank feathers that lightly encompassed the wings as the birds swam down through the clear water. They remained below 30 sec., more or less, then rose buoyantly as if suddenly released by a spring. Immediately on reaching the surface, the sea lettuce, which hung pendent from their bills, was shaken from side to side with vigorous movements of the head. This head movement was repeated numerous times until the sea lettuce was swallowed.

In the vicinity of the coast cities, greater scaups frequent the waters at the outlets of sewers and return to these places year after year. They are constant visitors also to the boat slips at city wharves where, in company with several species of gulls, they feed upon scraps thrown from the ship galleys. In some harbours there invariably is a flock in regular attendance until the spawning of herring in the spring attracts them elsewhere.

In waters where shooting is not permitted they show varying degrees of fearlessness of man. An extreme example is reported from Victoria where in a sheltered lagoon a fisherman captured by hand and banded a female that,

on hearing a certain call, was accustomed to swim up to him and be fed. This banded bird returned to the same locality for four successive winters (1937-1940) and each year was handled by the fisherman.

During the months of February and March there is a certain amount of courtship activity that increases as the season advances. Birds that have been feeding suddenly commence to bow, thrusting the head forward with neck arched until the tip of the bill touches the surface, then raising the head until the bill is in a vertical position or pointing slightly backward. Or the action may be less vigorous, the bill dropped gently to the surface, then raised slightly above the horizontal as in the action of drinking.

FOOD SUMMARIES, GREATER SCAUP DUCK

In the coast region 13 specimens were taken on fresh water and 21 specimens from a salt water habitat; the food is summarized under these headings. The number of specimens examined is indicated by the figure following each of the months represented.

Fresh water habitat—Fraser River, January, 1; March, 1: Cowichan River, December, 3; January, 3; March, 1: Chemainus River, November, 2; December, 1: Lake, Pender Island, March, 1.

Salmon eggs. Two specimens taken in November from the Chemainus river contained salmon egg cases; this was the only food item in one and 75% of the total contents of the other.

Salmon flesh. Broken down salmon flesh was the sole item in one and represented 65 and 95% of the contents of two other stomachs.

Amphipod. Three specimens of *Gammarus limnaeus* represented minor items in a specimen from Pender Island.

Caddis. Encased caddis larvae were the chief item in a December specimen from Chemainus river.

Other insects. More than 100 aquatic dipterous larvae and Tipulidae, made up the chief item in a Pender Island specimen that also contained several Formicidae and Gyrinidae.

Fresh water molluscs. Twenty-five specimens of *Sphaerium* sp. constituted 9% of the contents of a specimen from Pender Island, which also contained *Planorbis* sp. and another gastropod. Fragments of *Pisidium* sp. was the only evidence of food in a Cowichan River specimen.

Marine molluscs. Marine gastropods, remains of an earlier feeding at sea, were present in two stomachs.

Seeds. Seeds of *Carex exsiccata*, and a few seeds of *Rubus* sp., were the only items in one stomach from the Fraser River. Seeds of *Carex* sp. formed the entire contents of one, 40% of a second and 1% of a third, specimen from the Cowichan River. Seeds of *Scirpus americanus* in two stomachs and seeds of *Myriophyllum spicatum* and *Potamogeton* sp. in another were minor items.

Miscellaneous vegetation. Stems of a narrow-leaved grass formed the sole contents of one and 96% of a second specimen from the Cowichan River. Comminuted vegetable matter was the chief item in a specimen from the Fraser River.

Summary.—Thirteen Greater Scaup Ducks taken on fresh water in the coast region had eaten bulrush and sedge seeds and miscellaneous vegetable matter. Salmon eggs, which occurred twice, and salmon flesh, which occurred three times, are more important foods than these analyses indicate. Insects and molluscs were minor items.

Salt water habitat. Gulf of Georgia, January, 2; March, 1: Cowichan Bay, January, 1: Chemainus river mouth, November, 2: Comox, August, 1; March, 2: Departure Bay, January, 3; February, 7; March, 2.

Herring eggs. Opaque herring eggs were the chief item in a March specimen from Departure Bay.

Hydrozoa. The gullet and stomach of a specimen from Departure Bay were filled with a mass of hydroid colonies.

Crustaceans. A small kelp crab, *Pugettia producta* (Randall) and one hermit crab, *Pagurus* sp., constituted 8% of the contents of a well filled stomach of a bird from Departure Bay. *Hemigrapsus* sp. and undetermined crustacean fragments were the chief items in another and a third stomach contained one amphipod.

Molluscs. Gastropods occurred in 17, blue mussel in 6, limpets in 4, and barnacle scutes in 6 of the 21 stomachs examined. *Littorina* was the exclusive item in two, and predominated in three, other well filled stomachs. In one, the number was estimated to be 800 and in another 400 small specimens. Molluscs, chiefly gastropods, were the only item in six, and represented 70% of the contents of one other, stomach. Species identified: *Alectrion mendicus* Ild., *Columbella gausapata* Ild., *C. permodesta* Dall, *Bittium eschrichtii* (Medd.), *Littorina scutulata* Ild., *L. stichana* Phillippe, *Lacuna divaricata* (Fab.), *L. variegata* (Carp.), *Acmaea scutum* (Esch.), *A. pelta* (Esch.), *Margarites pupilla* (Ild.), *M. lirulata* (Carp.).

Eel grass. A small amount of eel grass, *Zostera marina*, was present in one stomach and probably had been taken with herring eggs which were also present.

Marine algae. Sea lettuce occurred seven times with an average percentage volume of 28.1. In one specimen it was the exclusive item. An unidentified algae constituted 20% of the contents of another stomach.

Summary.—On salt water by far the most important food is molluscs; the largest whole specimen found (*Alectrion mendicus*) measured 12 mm. Sea lettuce is the food of second, and crustaceans of third, importance. Herring

eggs were noted only once and undoubtedly this was owing to the fact that only one scaup was collected on the spawning grounds.

In Table I is given a food summary of the Greater Scaup Duck in the Okanagan and coast regions of British Columbia.

ECONOMIC RELATIONS

In the interior of British Columbia the Greater Scaup Duck is a species of some importance from the standpoint of wildfowling. It is relatively abundant, it decoys well, affords flight shooting in some localities, and it is an excellent table bird. Its food habits would seem to be related to other interests only to the limited extent to which it consumes aquatic insects and is a food competitor of trout or other commercially valuable fishes.

On the coast region its life history for a short time is involved with that of the Pacific herring and with the various species of salmon. So far as known this relationship works only in the interests of the ducks that feed upon the eggs of all these fishes. Any considerable reduction of this food supply might eventually reduce the number of ducks. To what extent, if any, the egg-eating habit of the species adversely affects the propagation of salmon and herring is not known. Most of the salmon ova eaten are drifting eggs of which an unknown percentage is considered to be infertile; the herring eggs taken as food are those that are unattached and of which a large proportion is washed ashore and becomes a waste product. This is discussed in some detail by Munro and Clemens (12, 13) and Munro (10).

The Greater Scaup Duck is not sought by hunters on the coast region chiefly because its flesh is generally considered unpalatable.

Lesser Scaup Duck

DISTRIBUTION ON THE PACIFIC COAST

Summer

The breeding range of the Lesser Scaup Duck in British Columbia has not been mapped in detail. In the southern half of the province it is included in the region lying east of the Cascade Mountains, west of longitude 120° W. and north of latitude 50°. It is a scarce breeder in the Okanagan Valley, which is the extreme southern limit of its breeding range in British Columbia west of the Rocky Mountains, more plentiful in the Kamloops and Nicola regions, and abundant in the Cariboo district. Information is lacking concerning nesting populations in the northern part of the province except in the Atlin district where it has been recorded as a common summer visitant by Swarth (16).

The Lesser Scaup Duck is known to nest commonly in that part of the province west of the Rocky Mountains known as the Peace River district (2). This region is associated geographically with the prairie country and its lesser scaup population may be isolated from any other within the political boundaries of the province, with the possible exception of the Atlin district where the fauna has marked eastern affinities.

TABLE I
FOOD OF GREATER SCAUP DUCK, AVERAGE PERCENTAGE VOLUME

Locality	Number of specimens	Salmon eggs	Salmon flesh	Herring eggs	Unidentified fishes	Crustaceans	Insects	Molluscs	Hydroids	Chere	Marine algae	Miscellaneous vegetation	Miscellaneous seeds
Okanagan Lake	68				0.03	0.01	1.65	6.08		72.00		19.65	0.58
Swan Lake	11						13.05	.36				16.50	70.09
Fraser River	2											45.00	55.00
Cowichan River	7		36.42				32.67	14.85				28.00	20.73
Chemainus River	3	56.67					62.00	30.00					1.66
Lake, Pender Island	1					1.00		100.00					7.00
Gulf of Georgia	3							100.00					
Cowichan Bay	1							100.00					
Chemainus River (mouth)	2					3.00		97.00					
Comox	3					33.00	.33	53.34			13.33		
Departure Bay	12			4.17		.67		60.58	8.17		23.50	2.91	

North of British Columbia it has been found nesting in southeastern Alaska (3), in east central Alaska (14), in the Yukon Territory (1). In none of these localities is it reported to be common.

So far as known it has not been recorded as nesting south of British Columbia in the State of Washington; there is one record for eastern Oregon and it is reported to nest abundantly in western Montana*.

Winter

In collections of scaup ducks from British Columbia, few winter taken specimens of *N. affinis* are represented. Thus of 54 specimens taken in January and February at Okanagan Landing, only three were of this species (January, 1931) and, of 32 collected in these months at various coast points, two were *N. affinis*. Evidence of the comparative scarcity of the species in winter is borne out by field observations. There have been many opportunities to examine flocks of scaup ducks at close range and none have been identified as the small species. It seems fairly well established that the Lesser Scaup Duck is a scarce winter visitant in British Columbia.

In Washington state it is reported† to be much less common than *N. marila* and restricted almost entirely to a fresh water habitat; no reference is made to its status in winter.

In the Portland region, Oregon, it is the most abundant of the wintering diving ducks, (6), and at Netarts Bay in December, 1912, and January, 1913, outnumbered all other ducks (5). It is said to be the commonest duck during the winter on salt water bays and marshes in California (4).

It is probable that the Oregon and California winter populations are much larger than the total summer populations of British Columbia and include migrants from various widely separated nesting grounds, including some lying east of the Rocky Mountains.

SEASONAL MOVEMENTS

Spring Migration

By what route the summer population of lesser scaups that presumably have wintered in Oregon and California reach British Columbia has not been determined; as the species becomes common in the lower Fraser Valley in late March or early April, it seems probable that one flight takes place along the coast. Thus near Chilliwack on March 29, 1934, flocks with a total of 300 birds were present on sloughs and backwaters of the Fraser River. From this point they probably travel northeast following the general direction of the Fraser and Thompson Rivers. At any rate they appear in the Kamloops and Nicola regions early in April and a migration continues thereafter for a month or six weeks. The time of their greatest abundance is the latter part of April, then flocks congregate on certain lakes where amphipods are plentiful. For example, on Napier Lake, in the Nicola district the following

* Letter, Bureau of Biological Survey, Washington, D.C., March 6, 1940.

† Pp. 797-798 in *Birds of Washington*, Vol. 2, by Dawson, W. N. and Bowles, J. H. The Occidental Publishing Company, Seattle, Washington. 1909.

counts were made: 160 on April 25, 1934; 385 on April 27, 1938; 175 on April 25, 1939; 271 on April 22, 1940. The total on 12 small ponds in that vicinity on April 25, 1939, was 465.

At the same time a migration, thought to be composed of birds that had wintered on the lower Columbia River, was following the Okanagan Valley. Numbers congregated at several points, for example, in the Glenmore district, near Kelowna, and on Goose Lake, near Vernon, where the following counts were made: 271 on May 8, 1937; 250 on April 23, 1938; 70 on Goose Lake, April 21, 1939.

On the Arrow Lakes, Kootenay district, the species has been recorded between April 12 and June 3, and is reported to be common (7). May 3, 1933, is the earliest date of arrival at Atlin as recorded by Swarth (16). The numbers noted above are much smaller than those recorded for the Greater Scaup Duck on its spring migration and a comparison reflects the numerical status of the two species in British Columbia.

Autumn Migration

The return flight in autumn appears to follow the same course as that taken in spring. An exodus of adult males and yearlings of both sexes from the nesting grounds takes place soon after the flight feathers have been renewed in August. Very likely these birds make a long journey at the outset; at any rate, few are seen at this time in the Okanagan, which is close to the southern part of the principal nesting ground, or in the lower Fraser Valley. It is of interest to note that migrants have been observed in the Portland, Oregon, district as early as August 10, although the majority do not arrive there until mid-September (6).

In September, there is a second flight from the nesting grounds, of some part of the populations that have been disturbed by gunners. Finally, in October and early November, the third and largest movement takes place. Dates of their last appearance in the Cariboo region are not available but it seems probable that a few remain until the freezing of the small lakes, which normally takes place in early November. The latest record of appearance at Atlin is October 17, 1931, (16).

No information is available concerning a possible migration along the coast line north of the Fraser River. Kelso (7) refers to the species as common on the Arrow Lakes (August 13 to November 8) presumably the earliest and latest date on which individuals have been seen.

Three returns from a total of 29 Lesser Scaup Ducks banded at Buffalo Lake, Cariboo, British Columbia, are of some interest. The details are:

<i>Banded</i>	<i>Recovered</i>
Sept. 18, 1932.	Deer Lake, Ore., Nov. 9, 1932.
Sept. 14, 1933.	Fox Lake, Wisc., Oct. 27, 1933.
Oct. 2, 1933.	Odessa, Wash., Nov. 18, 1933.

REPRODUCTION

Arrival on Nesting Ground and Courtship

The Lesser Scaup Ducks become established on their nesting grounds in the Cariboo region during late April or early May, depending on the time the lakes become free of ice. At the same time, flocks of migrants may be present in other districts to the south. Thus on April 22, 1940, totals of 210 males and 60 females were counted on Napier Lake in the Nicola region. These were in four flocks and in each the members were widely scattered with few associated in pairs. This was a concentration of migrants on a rich feeding ground. On the other hand, a total of 174 on 105 Mile Lake, over 100 miles to the north, April 24, 1940, represented a breeding population plus the usual excess of non-breeding birds. Many of the 39 females present were paired and for the most part the mated birds kept apart from the numerous small groups that were scattered here and there over an area approximately one-half by one-quarter mile. Some of the small groups were thought to be composed chiefly of yearlings, others consisted of one or two females attended by a larger number of males and among these an occasional exhibition of courtship activity was observed. The groups of yearlings sometimes joined together, forming flocks up to 30 or so; these birds were restless and took flight at the least alarm, whereas the paired or courting birds showed less nervousness when approached.

On the same lake on April 27, 1939, the behaviour exhibited by a group consisting of one mated pair and two males was as follows: the mated pair swam slowly side by side with only a few inches separating them; the two other males followed several yards behind. Occasionally the mated male advanced in front of the female for two or three feet, turned and faced her for a moment, then resumed his position by her side. At other times he advanced only a few inches and crowded against her sufficiently to impel her to change her course. At such times she would turn about and the male would immediately press close to her again. Once one of the following males approached the female and she splashed over the water towards him which caused him to retreat.

At Storage Lake on May 10, 1939, a group of eight males and three females were in active motion on the water. The males encircled the females and swam towards them with heads retracted and breasts thrust out—they seemed actually to dance. Then several of the group would dive and the circle would be broken. Finally all would rise and fly swiftly about the lake, now high in the air now low over the surface, then splash into the water where they would reassemble in a circle and repeat the dancing courtship.

While the reproductive process is at full tide the males attend the females constantly and seldom leave them except when the latter are on the nest. When the female of a pair was shot and dropped to the water, the male immediately alighted beside it and for a few minutes, standing almost upright, splashed around the dead bird. Although previously wary it did

not fly until approached within 20 yards. When the male of a mated pair was shot the female flew straight away.

Nesting Grounds

Usually the nesting grounds centre about a lake of moderate depth that has bulrush growth on the shore and adjacent brushy coves. Those that contain an abundance of amphipods and aquatic insect larvae support the largest populations. The small grassy type of slough used by some surface-feeding ducks and the open alkaline sloughs and deep lakes frequented by Barrow's Golden-eye, *Glaucionetta islandica*, seldom attract the Lesser Scaup Duck. The two lakes described below are typical nesting habitats.

Lily Pad Lake is one and one-half miles long by 200 yards wide and is used as an irrigation reservoir, the water level being maintained by a dam across its outlet at the north end. The lake bottom is hard, the shores stony, and the peat-stained water deep to the shore line growth of dwarf birch, willow, and black spruce. These low-lying brush-covered areas adjacent to the lake are flooded during the summer thus providing additional cover for waterfowl; farther back from the lake lodge pole pine and poplar predominate.

The dominant vegetation in the main body of the lake is Yellow Pond-lily, *Nuphar*, and the surface is covered with the leaves of this plant. At the south end is an open bulrush marsh and at the north end a 50 acre marsh of the same type with several deep channels through it, terminating in a *Carex* meadow. Aquatic plant growth, other than *Nuphar*, reaches the maximum of abundance in these marsh areas and includes *Myriophyllum*, *Potamogeton pectinatus*, *P. natans*, *P. pusillus*, and *Polygonum amphibium*. Probably the most abundant vegetable food is the seed of *Scirpus* that is not available in abundance until the crop matures in late summer. The most plentiful animal food is an amphipod; leeches and snails (*Planorbis* and *Lymnaea*) also are numerous.

Tatton Lake is a valuable waterfowl nesting ground divided into three relatively deep areas separated by shallows grown over with heavy bulrush marsh. The entire length is approximately one and one-half miles and the maximum width three-eighths of a mile. The lake is entirely surrounded by open bulrush growth and in August the waters on the outside of this shoreline marsh are covered with filamentous algae in a matted deposit of varying thickness and width through which young ducks have difficulty in moving. On the bottom are dense *Chara* and *Nitella* meadows and this is the dominant growth; next in abundance is *Myriophyllum*. The combination of *Chara* and bulrush seems to be one well suited to a dense population of certain diving ducks and coots, the *Chara* providing an abundance of food in itself and in the various organisms it harbours, the bulrush providing adequate nesting cover.

Nesting

Egg laying probably begins in early June but the majority of nests examined have been found in July and August. The earliest date on which a female

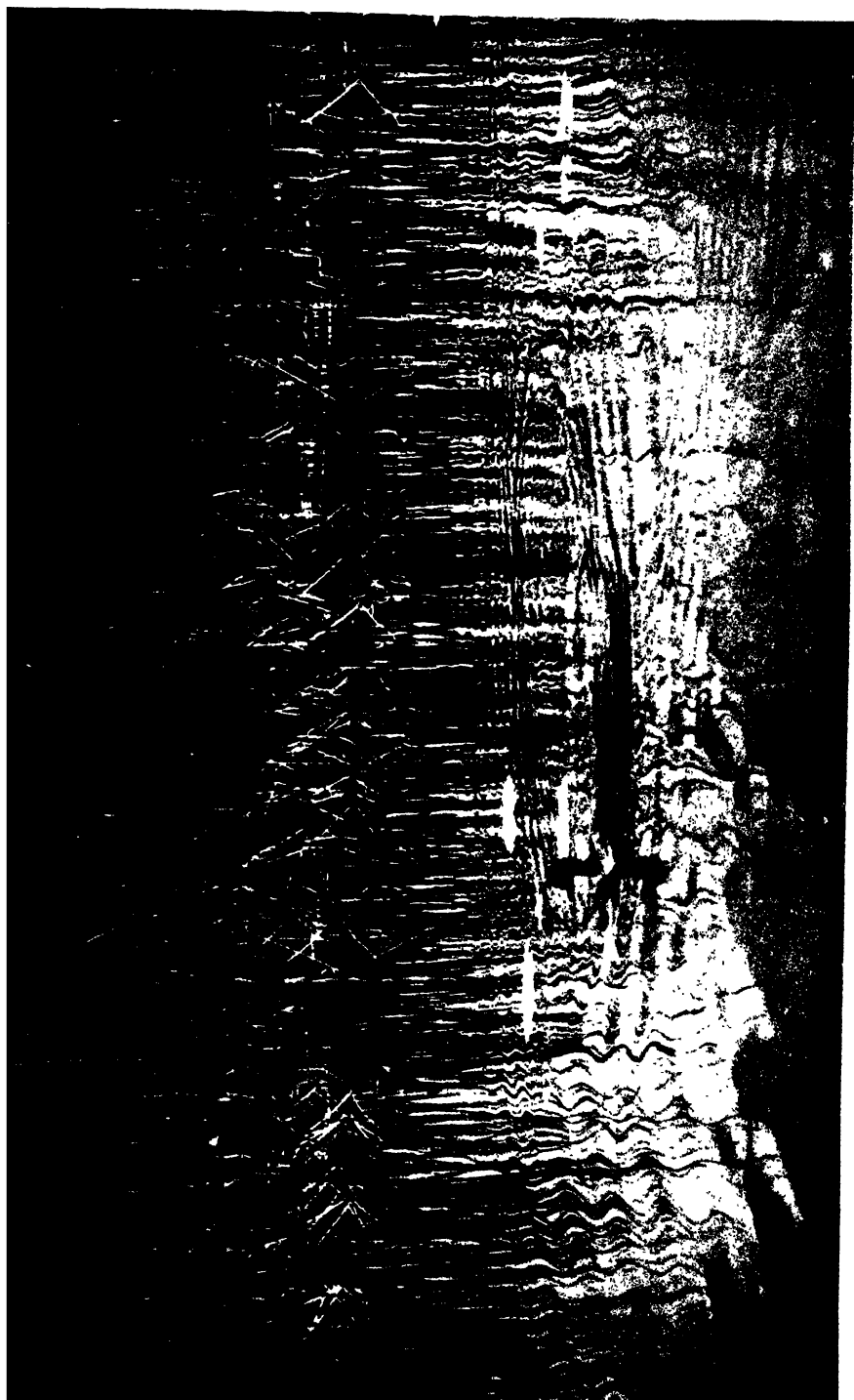


FIG. 1. *Lesser Scaup Duck swimming in front of nest site, Lily Pad Lake, British Columbia.*



FIG. 2. *Lesser Scaup Duck taking flight, Talton Lake, British Columbia.*

FIG. 3. *Nest and 15 eggs of Lesser Scaup Duck, 105 Mile Lake, British Columbia.*

was flushed from a nest is July 6 (Lily Pad Lake, 1938), the latest August 10 (Tunkwa Lake, 1939).

A variety of nesting sites are used, the most common being a dry situation under cover of one kind or another and within a few yards of a lake shore. Less frequently a wet, marshy site is chosen. The following sites, except that on the muskrat house, can be considered typical.

Lily Pad Lake—July 6, 1938. Three nests containing eggs ($\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$) on a narrow, stony peninsula covered with grass, one hidden in a pile of old boughs, another in a grass clump beside a large boulder, the third flush with the ground in an open situation and screened by grasses and *Potentilla*.

Tatton Lake—July 22, 1939. A nest of dry bulrush, 11 in. in diameter and three inches in height, on top of a flattened muskrat house in open bulrush marsh, contained seven eggs; no down had been added.

Watson Lake—July 24, 1939. A nest of dry and green bulrush, the top 10 in. above water in a thick clump of bulrush, contained seven eggs deeply imbedded in the nest material; no down had been added.

Tunkwa Lake—August 10, 1939. Each of four nests on a dry grassy island, 10 to 20 ft. from the water, contained seven eggs all well banked with down.

105 Mile Lake—August 10, 1937. A nest on a small rocky island under thick cover of Loco weed, *Astragalus canadensis*, contained 15 eggs almost completely covered by a mixture of down and rubbish (Fig. 3).

In some cases females when flushed from a nest fly off and do not return to the vicinity until the observer has left, in others they may alight on the water close by the nest and swim back and forth in obvious concern. The latter behaviour perhaps is exhibited chiefly by individuals in the early stages of the reproductive process. An extreme example of this in which a female walked off her nest then returned and defended it by grasping with her bill the fingers of an observer has been recorded by Munro (9). Almost invariably the female when flushed from the nest discharges excrement on the eggs. The voided matter carries a strong odour and the action probably has some protective value.

Behaviour of Females and Young

The majority of the young appear from July 15 to August 10; the earliest date recorded for first appearance of a female with downy young is July 4 (Lily Pad Lake, 1938) and the latest August 23 (Tatton Lake, 1939). In both cases the young were a day or so old. Late broods of young with flight feathers ensheathed have been observed in late September, for example, at Fawn Lake on September 26, 1940.

On lakes containing large populations there is much mixing of broods and banding together of several families. Thus at Watson Meadow slough, July 20, 1937, seven females and 64 young birds of various ages were associated in one flock. The young seemed to attach themselves to the nearest female and during the hour these birds were under observation several changes in

the association of young with different females took place. At one time the population was divided as follows: broods of 8, 9, 15, each led by a female; three females with 25 young; 4, 2, and 1 young birds unaccompanied by females.

The mingling of broods and their joint care by several females is of regular occurrence; for example, at Straight Lake on July 15, 1938, three females accompanied a raft of 34 downy young, and on Tatton Lake, July 21, 1938, three females accompanied 35 downy young. In both instances, the young swam in grouped formation, the females sometimes ahead, sometimes a little to one side. At 150 Mile Lake, July 22, 1938, combined broods totalling in one case 22 and in the other 18 downy young were each accompanied by two females. At the same place on July 17, 1939, a band of 55 downy young was attended by three females, one leading the band and two following in the rear or at the side. The young at times swam in a long line, some singly, others two or three abreast; again they would crowd together in a compact group. Many other instances of communal care of young could be cited.

Upon the appearance of a canoe on their territories, females exhibit, in various degrees, concern for the safety of young. The most common reaction to alarm is for the female to swim rapidly towards the canoe, sometimes flat on the surface, again on her side so that the white underparts are exposed. She surges across the surface in one direction then in another and threshes the water with her wings. Sometimes this is accompanied by a soft, purring note.

When two or more females with combined broods are alarmed, it is usual for one to demonstrate in this manner whereas the other female, or females, leads the broods to another part of the lake or into marsh coves. This behaviour has been observed many times. The demonstration on the water may last for only a few minutes or it may be continued at intervals for a much longer time. Thus at Cummings Lake, August 10, 1940, the behaviour of two females with a combined brood of 17 was as follows: one female led the young, which were alarmed and scampered over the water, into a narrow marsh channel where they disappeared from view; the second female rushed across the water towards the stationary canoe and continued to swim back and forth a few yards from it. When the canoe was turned about and paddled in a direction opposite to that taken by the young, she preceded it for a quarter of a mile in a series of short flights. At the end of each flight she splashed across the water near the canoe in the usual manner.

In the early part of the season, defence behaviour follows this general pattern very closely. One of the several exceptions noted was observed at Abel Lake, July 23, 1918, where two females that accompanied a total of 25 young did not react in this way. Both left the young, which were travelling in single file along the outer edge of the Yellow Pond-lily growth that encircles the lake, alighted on the water 50 yards or so in advance of them and, after swimming a few yards, again took to wing and left the vicinity. Meanwhile the combined broods, still in single file, continued on their course for 20 yards

or so, then turned at right angles and scampered over the lily-pads, swimming and running, to disappear in the thick *Scirpus* growth. At no time did the adult females exhibit any of the usual maternal reactions to alarm.

Maternal care becomes less marked later in the summer. Thus at Brigade Lake (August 7, 1939) only two of the six females present, with broods, reacted to alarm in the familiar manner. So also at 105 Mile Lake on August 22, 1939, females showed less concern for their young than had other females under similar circumstances three weeks earlier. On the latter date, each of eight broods, with a total of 58 birds ranging from downy young a few days old (two broods) to one brood about one-third grown, was accompanied by a single female. All but one of the females flew to another part of the lake on being disturbed. When left thus these young either ran over the water or dived, whereas young defended by the female usually swim off with little manifestation of alarm.

In general, it may be said that as the summer advances females exhibit more concern for their own safety and less for that of their young in accord with changes in metabolism as the reproductive process nears its seasonal conclusion.

Mortality of Young

The Lesser Scaup Duck because of its late breeding, when heavy cover is available to shield the nests, appears to suffer less from attack by predators than do some other ducks. The number of young surviving to late summer is higher than with other species and it is not uncommon to find a well grown brood in late August numbering 10 or more.

A primary factor in the relative safety of adolescents is their habit of mingling with bands of moulting adults and yearlings. The older birds are exceedingly wary and when alarmed their behaviour follows a consistent pattern of high protective value as will be explained in the following section. The young are quickly influenced by the actions of the older birds and their reactions to alarm are similar. Another factor of survival value is the generally protective attitude of the female towards the young.

Possible destruction by coots, or by loons and Holboell's Grebe has been discussed by Munro (10). Such loss as may be caused through attack by these birds does not seem to be a factor of importance.

Evidence that Horned owls, *Bubo virginianus*, kill females on the nest has been obtained but such instances appear to be uncommon.

Perhaps one of the chief losses is through drowning or suffocation brought about when the small young become entangled in weeds or matted deposits of filamentous algae, which are abundant on some of the lakes they inhabit. In most cases of this kind observed, it was considered that the small birds had been washed into the heavy growth during rough weather or had dived through an opening in the blanket of algae and been unable to emerge.

Summer Populations

The continued association of lesser scaups in flocks for some time after their arrival on the nesting grounds has been referred to. The size of these flocks begins to decrease in early June as mated pairs move out to nest elsewhere in the general vicinity and for a time the residue may be chiefly yearlings of both sexes, none of which are in breeding condition. A few paired adults sometimes are present as late as July. For example, at Tatton Lake (July 5, 1938) a total of 55 consisted of 10 mated pairs and 35 yearling males. All the mated birds finally disappear and shortly afterwards the population increases again with the return of the first post-breeding males. The number of the latter grows almost daily and in early August adolescent birds that have been reared on the lake also join the company. Thus a favoured lake may finally harbour a large proportion of the local population. At this time the adult males and yearlings of both sexes are moulting their flight feathers and when the moult is completed many move elsewhere, so that constant changes take place in the size of the population. This may be illustrated by an account of conditions at 105 Mile Lake, which is small enough that enumerations are comparatively easy.

On July 13, 1939, this population was composed of 200 adult and yearling males, and 12 yearling females; on July 24, the number had increased to an estimated 260 of which about 40 were flightless; on August 22, the raft consisted of 50 adult and yearling males, 70 adult and yearling females and 50 well grown young. About 20 adults were in flying condition. Elsewhere on the lake on July 24, and later, were females with young broods that kept apart from the older age groups.

Similar observations have been made elsewhere numerous times. Thus at Tatton Lake on August 23, 1939, a smaller association, consisting of 60 males, 40 females, and 70 well grown young, was studied. Most of the old birds were flightless; the largest young had flight feathers ensheathed. There was no difference in behaviour as between flightless adults and young except that the latter were less wary. When disturbed all dived and swam under water to reassemble on a distant part of the lake. Few adults appeared above the surface after the initial dive. Those that did were visible only for an instant before they again dived, sometimes sending a jet of water two or three feet in the air at the moment they disappeared.

Again at Minnie Lake (August 10, 1939) approximately 500 lesser scaups, chiefly adult males and yearlings of both sexes, formed part of a raft which included other species of diving ducks. These birds were exceedingly restless; flightless birds swam long distances under water, and those capable of flight took wing, at the slightest alarm. Thus on this lake, which is a comparatively large one, it was not possible to make satisfactory counts or to determine the various age groups.

Table II sets forth a number of typical summer populations, some restricted to females with young, others including non-breeding yearlings and post-breeding males.

TABLE II
SUMMER POPULATIONS, LESSER SCAUP DUCK

Locality	Date	Adults, ♀, with brood	Young	Adults, ♂, yearlings, ♂	Adults, ♀, yearlings, ♀
150 Mile Lake, 25 acres	July 31, 36	7	70	0	0
150 Mile Lake, 25 acres	Aug. 2, 37	5	57	10	27
150 Mile Lake, 25 acres	July 22, 38	9	85	17	7
150 Mile Lake, 25 acres	July 17, 39	3	55	7	0
150 Mile Lake, 25 acres	Aug. 10, 40	6	64	0	0
149 Mile Lake, 10 acres	July 31, 36	7	64	0	0
149 Mile Lake, 10 acres	July 12, 38	0	0	25	0
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 31, 36	8	63	175	25
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	June 4, 37	0	0	6	6
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	Aug. 6, 37	1	13	(Adult)	(Adult)
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 3, 38	0	0	375	300
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 26, 38	5	45	210	40
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	Aug. 6, 38	7	60	70	30
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	Aug. 16, 38	8	65	90	43
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	April 25, 39	0	0	140	60
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 13, 39	0	0	120	80
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 24, 39	3	41	200	12
105 Mile Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	Aug. 22, 39	11	108	210	50
Watson Meadow Lake, 75 acres	July 20, 37	7	64	50	70
Straight Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 15, 38	7	78	0	0
Brigade Lake, $1 \times \frac{1}{2}$ mi.	Aug. 7, 39	6	72	100	20
Tatton Lake, $1 \times \frac{1}{2}$ mi.	July 22, 39	3	17	60	12
Tatton Lake, $1 \times \frac{1}{2}$ mi.	Aug. 23, 39	15	111	60	28
Lily Pad Lake, $1\frac{1}{2} \times \frac{1}{2}$ mi.	July 21, 39	4	20	50	14
Swan Lake, Okanagan	July 31, 40	2	16	0	0

Moult and Plumages

Interest in the plumage sequence of the Lesser Scaup Duck is stimulated by study of the summer populations, where a wide variety of plumage comes under observation.

When the flocks first arrive on the nesting grounds the males of the previous year may sometimes be distinguished from adult males by their generally darker appearance owing to the admixture of grey on the white areas. This distinction becomes more pronounced in early July when young males are rapidly going into an eclipse and the adult males are still in breeding dress (Fig. 4). For example, it was noted at Tatton Lake on July 8 that in a population consisting of 10 mated pairs and 35 yearling males, the former could readily be distinguished by their conspicuous white flanks. Later as adult males eclipse, this distinction disappears and, in August, with occasional exceptions, all look alike.

In the spring, the distinctive white areas at the base of the bill on the female is apparent in most individuals and in the field those of the previous years cannot readily be distinguished from adults. Later the rich brown of the breeding female wears to a lighter, more golden, shade; in some the white

area at the base of the bill is entirely obscured; in others it is retained and in some a second white patch appears on the cheek (Fig. 5). In June most of the incubating females lack the white areas on the face. Thus at Tatton Lake, June 11, 1940, one in 16 mated females had conspicuous white cheek patches.

Meanwhile a rapid fading is taking place in the plumage of the yearling females so that by July these non-breeding birds are much lighter in appearance than the adult females with broods. There is no uniformity in the progress of the plumage disintegration and no two individuals appear exactly alike (8). The difference in coloration between adult and yearling females was illustrated at 105 Mile Lake (August 6, 1938), when a flock of seven yearlings and one adult swam past in regular alignment. The yearlings were faded on neck and head to a pale buff or cinnamon whereas these regions on the adult were rich brown in contrast.

In late August and September young males, as seen under the usual conditions of field study are darker than young females and show more conspicuous white areas about the base of the bill (Fig. 4). This marking is absent in some young females but apparently is always present in the newly moulted adult and yearling females. However, at this time the amount of individual variation among both adults and young is too great always to permit satisfactory field identification of sex and age groups.

Sex Ratio

In flocks of spring migrants that contain adults and yearlings of both sexes there is usually an excess of males over females in the ratio of four or five to one. Examples: Napier Lake, April 22, 1940, 210♂, 61♀; 103 Mile Lake, April 23, 1940, 60♂, 20♀; 105 Mile Lake, April 23, 1940, 135♂, 39♀; Sepa Lake, April 24, 1940, 50♂, 15♀. On the nesting grounds in early summer, where concentrations of non-breeding birds, chiefly yearlings, take place, the excess of males is even greater. At this time the picture is obscured because such flocks may contain some adult males that have bred earlier in the season and may lack a corresponding number of adult females that are incubating eggs or caring for young. Furthermore, the number of yearling females present may represent less than the actual population of this age group; because of the excess of males over females, some yearling females may breed and thus not be present in these associations. However, taking all this into account, there does appear to be a very definite male preponderance in the sex ratio.

FOOD STUDIES OF LESSER SCAUP DUCK

The Lesser Scaup Duck of any age appears to obtain most of its food by diving. In general the feeding habits are similar to those of the Greater Scaup Duck except that the former seems more inclined to keep moving fairly rapidly as it feeds. Thus on a narrow, deep lake about a mile in length a flock was seen to fly down-wind and alight near the end of the lake. Here they separated and immediately afterwards started swimming up the lake against the wind. Every few minutes they dived, slipping under water

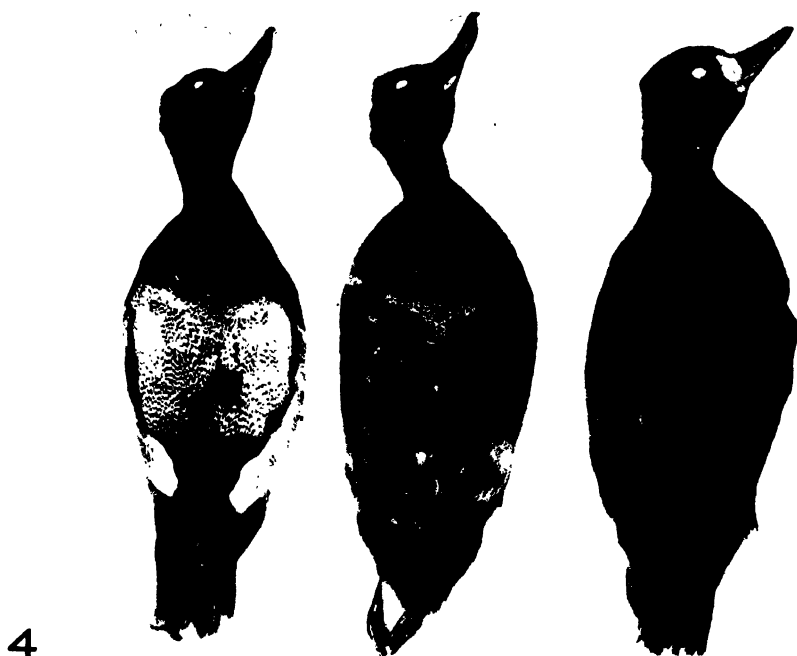


FIG. 4. Dorsal view, male Lesser Scaup Ducks; left to right—adult, winter and breeding plumage (June 13); eclipse (August 6); approximately two months old (September 25).

FIG. 5. Dorsal view, female Lesser Scaup Ducks; left to right—adult, winter (December 5); adult, breeding (June 13); yearling, showing extent of summer fading (August 22).

quietly to reappear in from 10 to 20 sec. This manner of feeding and travelling was continued until the ducks had traversed the length of the lake.

The following section summarizes the food eaten by 57 Lesser Scaup Ducks taken in various localities in the interior and on the coast region of British Columbia; the number following each month indicates the number of specimens taken.

Food of downy young—Cariboo region, 150 Mile Lake, July, 1; Watson Meadow Lake, July, 1; 105 Mile Lake, August, 4.

Amphipods. Fragments of amphipods occurred in all three and was the main item in one.

Aquatic insects. A chironomid larva was a minor item in one, Corixids occurred in another, and unidentified insect fragments represented 39% of the food in a third specimen.

Coleoptera. Fragments of a terrestrial beetle constituted the chief item in one stomach.

Molluscs. Shell fragments were present in one specimen.

Seeds. The chief item in one stomach was seeds of *Potamogeton pectinatus*, *Eleocharis palustris*, *Polygonum* sp., *Scirpus* sp.

Food of older young—Cariboo region, Tatton Lake, August, 2; September, 2; 105 Mile Lake, August, 1; September, 4.

Amphipods. Comminuted amphipods formed 80 to 100% of the food remains in seven stomachs.

Aquatic insects. Corixids and fragments of unidentified species were minor items in four specimens.

Hymenoptera. Ants formed one-half the contents of one stomach that was less than one-quarter filled.

Seeds. *Scirpus* seeds were present in four and were the exclusive item in one bird.

Summer food of adults—Cariboo region, Tatton Lake, June, 2; August, 1; 105 Mile Lake, August, 6.

Amphipods. Amphipods were present in the nine specimens and composed 95 to 99% of the contents of seven; in one, approximately 200 specimens of *Gammarus limnaeus* had been eaten.

Aquatic insects. Corixid fragments were minor items in three and *Odonata* nymphs occurred in two specimens.

Coleoptera. Fragments of a terrestrial beetle were present in one stomach.

Seeds. A small number of seeds were present in all but one of the specimens examined, the species represented being *Scirpus* sp., *Myriophyllum spicatum*, *Potamogeton pectinatus*, *Polygonum amphidium*.

Miscellaneous vegetable matter. Unidentified plant material was present in one specimen.

Autumn food—Cariboo region, Disputed Lake, 1; Longbow Lake, 5; 103 Mile Lake, 1; 105 Mile Lake, 2; Fawn Lake, 12 (representing period September 23–October 6, 1940).

Amphipods. Amphipods were found in 14 of the 21 specimens in this group; in one-half this number they represented 90 to 100% of the total stomach contents, in two others 50 and 60%, in the remainder two to five per cent. One specimen contained over 180 specimens of *Hyalella azteca*.

Aquatic insects. In one stomach *Odonata* nymphs constituted the exclusive item and in another represented 75% of the total contents. Corixids occurred as small fragments in seven and caddis in two stomachs.

Molluscs. A single *Planorbis* was present in one specimen.

Miscellaneous seeds. Seeds of *Eleocharis palustris*, *Scirpus* sp., *Potamogeton pectinatus*, *Potamogeton heterophyllus*, *Myriophyllum spicatum*.

Miscellaneous vegetation. This item was third in total percentage volume and consisted chiefly of comminuted vegetation. Several large fragments were identified as stems of *Potamogeton* sp.

Autumn and winter food—Okanagan Region.

Okanagan Lake, January, 3.

Aquatic insects. A caddis larva and fragments of a Corixid were present in one specimen.

Chara. *Chara* was the exclusive item in one well filled stomach.

Miscellaneous vegetable matter. Comminuted vegetable matter was the exclusive item in 1 and 80% of the total in another stomach. *Scirpus* seeds were present in one specimen.

Swan Lake, October, 1; November, 3; December, 4.

Amphipods. Six specimens of *Hyalella azteca* formed a minor item in one stomach.

Aquatic insects. Eighteen damsel fly nymphs and 31 whole Corixids, plus fragments of both, formed the largest item in one well filled stomach. Corixid fragments were present in four other specimens and microcaddis larva cases in two. Chironomid larvae constituted in one specimen 85% and in another four per cent of the total food, the numbers present being 30 and 10 respectively. Two Gyrinid beetles were present in one specimen.

Molluscs. Opercula of small gastropods were present in two and small *Planorbis* in a third stomach, in each case representing a small percentage of the food.

Leech. Leech egg cases were found in one stomach.

Miscellaneous seeds. Seeds of *Ceratophyllum demersum* were the chief item in each of four stomachs that otherwise contained little food. Seeds of *Scirpus americanus* were present in one, *Potamogeton pectinatus* in a second, and *Potamogeton* sp. in each of two other stomachs.

Miscellaneous vegetable matter. Tubers, leaves, and winter buds of *Potamogeton pectinatus*, together with unidentified plant material, composed 39% of the contents of the October taken specimen. Unidentified plant material formed slightly less than one-half of the contents of a second and was the chief item in a third stomach.

Winter food—Coast Region: Fraser River, November, 2; Cowichan Lake, December, 1; Chemainus River, December, 1.

Insects. One *Odonata* nymph was in the Cowichan Lake specimen.

Molluscs. Comminuted mollusc shells were the only evidence of food in the Chemainus River specimen; one specimen of *Planorbis* and one of *Pisidium variable* were in the Cowichan Lake specimen.

Seeds. Seeds of *Potamogeton pectinatus* were present in two, and seeds of *P. heterophyllum* and *P. foliosus* in one, of the Fraser River specimens.

Miscellaneous vegetable matter. Comminuted plant material was the chief item in two specimens from the Fraser River.

Summary.—As may be seen from Table III, amphipods predominated in the food of all age groups from the nesting ground in the Cariboo Region; seeds of aquatic plants were next and aquatic insects third in importance. *Chara* and other plant material were the chief food of three winter taken specimens from Okanagan Lake; plant material and insects predominated in the food of eight late fall and early winter specimens from Swan Lake; on the coast molluscs and vegetable matter were represented in about equal proportions.

TABLE III

FOOD OF LESSER SCAUP DUCK, AVERAGE PERCENTAGE VOLUME

Locality	Number of occurrences	Amphipods	Insects	Molluscs	Leech	Miscellaneous vegetation	Miscellaneous seeds	<i>Chara</i>
103 Mile Lake	1	5.00					95.00	
105 Mile Lake	14	62.72	18.35			0.36	18.57	
Tatton Lake	7	72.00	8.28	10.00		.14	9.58	
150 Mile Lake	1	95.00	5.00					
Disputed Lake	1	100.00						
Longbow Lake	5	79.80	20.00				.20	
Fawn Lake	12	26.58	18.67			29.59	25.16	
Watson Meadow Lake	1		39.00	1.00			60.00	
Okanagan Lake	3		6.00			60.00	.67	33.33
Swan Lake	8	0.12	22.00	.63	0.63	22.25	54.37	
Cowichan Lake	1		50.00	30.00			20.00	
Lower Fraser River	2					96.50	3.50	
Chemainus River	1			100.00				

ECONOMIC STATUS

The remarks made with reference to the value of the Greater Scaup Duck as a game bird apply equally to *N. affinis*.

Animal food eaten during the summer on the nesting grounds is composed chiefly of amphipods and aquatic insects, both valuable food for trout, and in some few localities the Lesser Scaup Duck and trout might compete for such food. By far the largest amount of nesting, however, is on shallow, muddy, or alkaline waters that are not inhabited by trout. Consequently such competition as may take place is of little economic importance.

The Lesser Scaup Duck has not been found feeding on the eggs of either salmon or herring but it seems likely that some of the scaups that visit salmon streams in the late fall are of this species.

Acknowledgments

Grateful acknowledgment is made to the Bureau of Biological Survey for permitting the use of their original records of stomach analyses of specimens collected, chiefly by the author, during the years 1911 to 1922; to Dr. Clemens for the co-operation mentioned; to the Fisheries Research Board of Canada for the use of laboratory facilities; to the National Museum of Canada and the Provincial Museum of British Columbia for various records; to the Entomological Branch, Department of Agriculture, Ottawa, Canada, and to various members of the staff of the Pacific Biological Station for the identification of certain food items.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

MAY, 1941

NUMBER 5

RED SORE DISEASE OF PIKE¹

By G. B. REED² AND G. C. TONER³

Abstract

"Red sore" disease of pike, common in these fish in eastern Ontario waters, is caused by an infection with *Proteus hydrophilus*, the organism previously shown to be responsible for the widespread "red leg" disease of frogs and probably responsible for "ulcer disease" in trout in a New York hatchery.

A disease of fish, characterized externally by red areas to open necrotic lesions of the skin on any part of the body or fins, has been known for many years in eastern Ontario. The disease, generally described locally as "red sore", has been noted most frequently by both anglers and commercial fishermen in the pike, *Esox lucius*, but other species are said to show it to a less conspicuous degree. It has been described as occurring in fish of the St. Lawrence, Rideau, and Napanee river systems in eastern Ontario. Pike from some areas in these systems appear to be free of the disease, in other regions it is apparent on most of the pike taken.

There does not appear to be any published reference to the disease. Anglers, commercial fishermen, and fish experts have expressed the opinion that it does not occur in western Ontario or in Quebec. This may indicate that the disease has only a regional distribution or it may mean that in other regions it is of relatively rare occurrence.

The disease in pike is extremely varied. In its mildest form, it varies from reddened points in the skin suggesting slight petechial haemorrhage to deep red areas several square centimeters in extent, the scales frequently being displaced. In the more severe form it appears as red, slimy, necrotic areas extending through the skin and in some instances deep into the muscle. The visceral organs, in gross appearance, are unchanged or in some instances the kidney appears darker in colour and softer in texture than the normal.

Ten pike showing the more severe superficial lesions, taken from different lakes on the St. Lawrence, Rideau, and Napanee river systems were subjected to a rather detailed bacteriological examination in an attempt to determine whether or not the disease was of bacterial origin and, if so, to isolate the causal agent. The method adopted was to make a series of cultures from each fish as soon as possible after it was taken from the water. The time

¹ Manuscript received January 16, 1941.

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varied from 1 to 24 hr. After more extensive procedures with the first few fish, the method adopted was to scrape the superficial lesions and introduce the scrapings into meat infusion broth. The body cavity was then opened aseptically and similar cultures were made from body fluid, heart blood, spleen, liver, and kidney. The cultures were incubated for 18 to 20 hr. at 28° C.

Primary cultures from skin lesion scrapings always contained several species of bacteria, including those usually present in normal fish slime. Blood or viscera from recently killed fish usually yielded no viable bacteria or one species alone; from all the tissues of fish that had been out of water for a number of hours, mixed growths consisting of three or four species were frequently obtained. These primary cultures were plated and pure cultures prepared. As far as possible the species were identified. All were tested for pathogenicity in goldfish.

Cultures of *Proteus hydrophilus* were obtained from the 10 diseased pike examined i.e., from the skin lesions of all, from the heart blood of two, from the spleens of two, and from the kidneys of three. Attention was concentrated on this species because of its well known causal relationship to the widespread "red leg" disease of frogs and its known pathogenicity in fish experimentally inoculated.

Pathogenicity

Twenty cultures of *P. hydrophilus* isolated from skin lesions and viscera of 10 pike with "red sore" and from the blood of two frogs with advanced "red leg" have been tested for pathogenicity in goldfish and one was tested for pathogenicity in several other animals.

The cultures, grown for 24 hr. at 28° C. in nutrient broth, were injected intraperitoneally into goldfish in 0.01 cc. doses (0.1 cc. of a 1/10 dilution). Each of the 20 cultures killed the animals in 24 to 72 hr. Doses as low as 0.001 cc. of two of the 24-hr. cultures killed them in five to seven days but doses of less than 0.001 cc. produced no symptoms. In goldfish there was a considerable increase in body fluid. It was slightly viscous, brownish in colour, and contained large numbers of bacteria but there was no increase in cells. Pure cultures of the organisms were obtained from the body fluid, heart blood, and kidneys.

Doses of 0.01 to 0.1 cc. of a 24-hr. culture injected subcutaneously into goldfish caused the development of local necrotic lesions and the fish died in three to five days. The organisms were recovered from heart blood of the dead fish.

An attempt was made to produce infection by light scarification of the skin over an area from which four to five scales had been removed and swabbing the spot with undiluted broth cultures. The fish reacted irregularly; some developed local necrotic lesions in three to five days, others showed no reaction. The addition of 1 to 10 cc. of culture to 2-litre lots of water each containing two goldfish, always failed to produce infection.

Of a 24-hr. culture of *P. hydrophilus* that killed goldfish in doses of 0.01 cc., 0.5 cc. was injected intraperitoneally into a snake, *Thamnophis sirtalis*, which had been in the laboratory for two months. The snake died in four days but there were no gross pathological changes except a considerable increase in blood stained body fluid from which organisms, identical with those injected, were recovered.

The same culture of *P. hydrophilus* was injected subcutaneously into the thigh of frogs in amounts of 0.1 to 0.4 cc. All showed the typical symptoms of "red leg" and died in three to five days. Similar reactions occurred when the infecting organisms were obtained from naturally diseased frogs or pike.

Intraperitoneal injection of 0.1 to 0.5 cc. of the previously mentioned culture of *P. hydrophilus* into four white mice caused death within 18 to 48 hr. No gross lesions were detected but there was an increase in body fluid; it was blood stained but had no marked increase in the number of cells. Organisms, identical with those injected, were recovered from body fluid, liver, and spleen but not from blood.

It is evident that the strains of *P. hydrophilus* recovered from diseased pike are capable of producing fatal infections in goldfish, snakes, and white mice and that in frogs they produce typical "red leg."

Antigenic Structure

Rabbits were immunized with cultures of *P. hydrophilus* recovered from three pike with "red sore" and from one frog with "red leg". This frog was from a lot that had just been received from Quebec. Agglutinating serum of reasonably high titre was obtained in each case; the organisms from each source were agglutinated in serum dilutions of 1/640 and 1/2560. Ten cultures of *P. hydrophilus* recovered from different diseased pike and two from frogs with "red leg" were tested against each of the above sera. The results are summarized in Table I. It is apparent that three cultures from different tissues

TABLE I

AGGLUTINATION REACTIONS OF 10 CULTURES OF *P. hydrophilus*, FROM FOUR PIKE, ONE SUCKER, ONE GOLDFISH, AND ONE FROG, WITH FIVE ANTISERA. FIGURES INDICATE TITRE OF SERUM

Culture	Source	Antisera				
		A5-4	S1-5	R8	X2-38	L3-2
A5-4	Pike A	1/640	-	-	-	-
A5-3	Pike A	1/640	-	-	-	-
S1-5	Pike S	-	1/2560	-	-	-
R8	Pike R	-	-	1/2560	-	-
R10-3	Pike R	-	-	1/2560	-	-
R10-1	Pike R	-	-	1/2560	-	-
T1	Pike T	-	-	-	-	-
U2	Sucker	-	-	-	-	-
X2-38	Goldfish	-	-	-	1/2560	-
L3-2	Frog	-	-	-	-	1/640

of pike *R* are antigenically identical as are two from pike *A*. Other cultures are, as far as determined by agglutination, antigenically dissimilar.

This antigenic heterogeneity is in line with what has been observed in other species of the genus *Proteus* by Wenner and Rettger (8) and Taylor (7).

Moltka (5), however, demonstrated that swarming forms of *Proteus vulgaris* could be divided into three groups on the basis of floccular agglutination. It should be noted that the cultures of *P. hydrophilus* at the time of isolation from pike and frogs were in the *H* or swarming form, or, more frequently, in the *O* or non-swarming form. Under the conditions in which they were held in the laboratory, the *H* types rapidly changed to the latter. At the time the rabbits were immunized and the agglutinating antigens were prepared, all the cultures were in the non-swarming form.

***Proteus hydrophilus* and Its Distribution**

Proteus hydrophilus (Chester) Bergey is a small rod 1 to 4 μ long by 0.5 to 1 μ in diameter, occasionally curved or irregular in shape, non-sporulating, and Gram-negative. Individual colonies on nutrient agar are 3 to 5 mm. in diameter, frequently, but not regularly, amoeboid, smooth, translucent, usually stippled, and bluish-white in colour. Acid and gas are regularly formed in glucose, sucrose, maltose, and mannite. Lactose, xylose, salicin and inulin are not fermented. The methyl red reaction is positive. Hydrogen sulphide is formed, gelatine is rapidly liquefied, much indol is formed. The organism was first isolated by Sanarelli (6) in 1891, and described in detail by Emerson and Noris (2) and Klup and Lackman (4) as the causal organism of "red leg" in frogs. It is probable that insufficient work has been done on this species to definitely establish its systematic position. In several studies of the genus *Proteus*, particularly that of Wenner and Rettger (8), it is not mentioned.

Recently Fish (3) has isolated *P. hydrophilus* from "ulcer disease" in fingerling brook, rainbow, blackspotted, and lake trout in the Cortland, New York, hatchery. This description of "ulcer disease" as well as an earlier description by Calkins (1) indicates that it is very similar if not identical with what has been found in the pike. The principal difference appears to be that the infected areas in the pike are much reddened, whereas Fish, found the lesions in trout to vary from a grey-white induration of the skin to a deep red necrotic ulcer. This difference may be the reaction in a different species of fish or possibly to the fact that an earlier stage of the disease has been observed in the hatchery. There are no trout in the regions in which the disease in pike has been studied.

Fish's observation, together with a private communication from Dr. R. H. M'Gonigle of the Atlantic Biological Station, suggests that infections with this organism may be an important factor in rearing of fish in the hatchery.

Acknowledgment

The writers are indebted to the National Committee on fish culture for financial assistance.

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A NEW METHOD FOR WASHING PARAMECIA¹

By F. T. ROSSER²

Abstract

The freeing of paramecia from bacterial contamination by passage through five one-tenth millilitre drops of sterile media is demonstrated. Wash drops are pipetted into the centre of Petri dishes. Paramecia are transferred from drop to drop in a small loop of 0.127 mm. platinum wire. They are allowed to remain for at least 15 min. in each wash, with the exception of the third in which they are kept for about 4 hr. Animals can be seen without difficulty when light is focused on the drop in which they are swimming. Addition of vitamins in various concentrations to the medium and association of a number of sterile paramecia in one tube failed to promote divisions.

Introduction

The method of freeing paramecia from bacteria as described by Parpart (1) and modified by Kidder *et al.* (2) is a procedure involving considerable time and an appreciable amount of equipment. The object in undertaking the present study was to develop a simpler washing technique.

In the scheme followed a platinum wire loop sterilized by flaming was used to transfer paramecia through a series of sterile wash fluid drops (1/10 ml.) contained in Petri dishes. This procedure eliminated wash fluid containers and sterile capillary pipettes. Since the work could be done at a transfer table wiped over with a disinfectant, a hood was unnecessary. Furthermore, by improvement of the washing technique, half the number of transfers sufficed to obtain bacteria-free paramecia.

Methods

In practice the one-tenth millilitre drops of wash fluid were placed in sterile Petri dishes with a pipette. No serious difficulty was experienced by drops of this size spreading. The loop (diameter 1 mm.) was twisted at the end of a 2-in. length of 30 gauge platinum wire inserted in a bacteriological needle holder. To facilitate operations it was bent off at right angles to the straight wire which was bent again at right angles about one-half inch from the loop, thus forming a Z at the end of the wire. It was found convenient to work on the glass stage of a dissecting microscope from which the tube was removed, since light could be focused on the drop making the paramecia easily visible to the naked eye. By brushing the loop over or lifting it up under an individual, single animals were readily isolated and held in the film formed in the loop. The paramecium was released when the loop was touched to a wash drop allowing the animal to swim away. Successive transfers were accomplished in a similar manner.

¹ Manuscript received March 15, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as N.R.C. No. 988.

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The animals frequently swam into the twists of wire at the base of the loop making it difficult to transfer them. This trouble was overcome by using a fine wire (diameter 0.127 mm.) free from twists, with the loop diameter somewhat less than 1 mm. and the end of the wire just touching at the first 90° bend. This loop not only facilitated transfer but carried less of the contaminated fluid to the next drop.

By extending the time in each wash it was possible to reduce the required number of washings from ten or more to five. The procedure finally adopted was to place a number of individual animals in a corresponding number of individual drops. When the first animal had been washed for at least 15 min. the operation was repeated transferring all the animals, in the same sequence, to the next wash drop. In this way 18 or 20 paramecia could readily be transferred from one wash to another in less than half an hour. Animals were allowed to remain 4 hr. in the third wash. To prevent evaporation about 1 ml. of sterile wash fluid was run out around the edge of these plates.

Pringsheim's medium for *P. bursaria*, to which was added 0.5% Difco proteose-peptone as recommended by Loefer (3), was used for washing and culturing. As described later, various concentrations of vitamins were added to the culture medium to determine their effect on promoting divisions.

To test the efficacy of the technique, wash plates were poured with Difco beef agar as soon as possible after removing the animal and were incubated at 28° C. for three days before reading. Tube cultures of washed paramecia were incubated at 20° C. for six days following which 1 ml. of the culture medium from each tube was plated to determine bacterial contamination.

Washing Experiments

In the first experiment the effectiveness of the loop transfer method in freeing paramecia of bacteria was tested. The animals used were from a hay infusion culture inoculated seven days before with a few millilitres of pond water swarming with paramecia. Table I shows the results of washing 10 paramecia. They were allowed to remain about 2 min. in each wash drop with the exception of the fifth in which they were kept 5 hr. Each one was transferred from its tenth washing to a culture tube.

TABLE I

EFFECTIVENESS OF THE LOOP TRANSFER METHOD IN FREEING PARAMECIA OF BACTERIA

Number of paramecia washed	Wash No.										Cultures after 6 days at 20° C.
	1	2	3	4	5	6	7	8	9	10	
	Average number of bacteria per wash										
10	10,000	266	31	3	33	1	0.6	0.6	0.4	0.3	Sterile

Since the average bacterial count after the fifth washing was one or less, and the colonies that appeared could be regarded as air contaminants (the plate covers having been raised four times), it was concluded that the number of washings necessary to obtain freedom from bacteria could be reduced.

A second experiment was undertaken to determine whether bacteria could be eliminated by five washings and whether the 5-hr. decontamination period should be made in conjunction with the third or fourth washing. The paramecia used were from a pure line strain isolated from pond water two months previously and grown in hay infusion medium.

TABLE II
EFFECTIVENESS OF FIVE WASHINGS IN FREEING PARAMECIA OF BACTERIA

Para- medium No.	Held five hours in wash No. 3						Held five hours in wash No. 4					
	Wash No.					Cultures after 6 days at 20° C.	Wash No.					Cultures after 6 days at 20° C.
	1	2	3	4	5		1	2	3	4	5	
	Number of bacteria per wash						Number of bacteria per wash					
1	2300	400	40	0	0	Sterile	1000	24	1	35	0	Contaminated
2	600	275	18	0	2	Sterile	400	87	1	47	0	Contaminated
3	1600	21	24	0	0	Sterile	500	135	7	47	0	Sterile
4	1200	2	15	1	2	Sterile	600	0	0	39	1	Sterile
5	700	146	65	0	0	Sterile	1500	2	0	64	0	Contaminated
6	1000	56	56	0	0	Sterile	1700	340	8	49	0	Sterile
7	1000	2	115	0	0	Sterile	400	0	1	44	0	Contaminated
8	1100	98	80	0	0	Sterile	1200	2	3	74	0	Contaminated
9	600	3	78	0	0	Contaminated	1900	200	6	41	1	Contaminated
10	2000	10	34	0	0	Contaminated	1700	220	3	44	0	Sterile
11	900	475	68	0	1	Sterile	900	48	0	112	1	Sterile
12	2200	2	36	3	2	Sterile	700	0	0	13	0	Contaminated
13	600	36	72	0	0	Sterile	800	47	1	24	0	Sterile
14	400	0	58	0	0	Sterile	600	1	1	144	0	Sterile
15	1400	1	46	0	0	Sterile	500	425	4	29	2	Contaminated
16	1200	250	118	15	0	Sterile	700	53	0	30	0	Sterile
17	300	390	82	0	0	Sterile	700	57	1	90	1	Sterile
18	500	400	33	0	0	Sterile	500	0	0	30	0	Sterile
Total	19,600	2567	1038	19	7		16,300	1641	37	956	6	
Average	1089	143	58	1	0.4		906	91	2	53	0.3	

NOTE: Number of bacteria in parent culture: 52,000,000 per cc.

Table II shows that the best results were obtained when the decontamination period accompanied the third washing. The fact that the paramecium sometimes became trapped in the twisted wire at the base of the loop, necessitating a transfer of the entire drop, probably accounts for most of the variations in bacterial numbers. When the animal remained in the loop, transfer was accomplished with a minimum carryover of medium. It is obvious that numbers of adhering bacteria were shed in the first three washings, and since eight of the ten contaminated tube cultures were from paramecia

held 5 hr. in Wash 4 (the organisms in nine of the tubes appeared to be coccus forms), it seems clear that sufficient time had not been allowed in the individual washings for the animals to free themselves from adhering bacteria.

The third series of experiments was designed to compare the number of bacteria transferred in the medium with the number transferred by the animal. Single paramecia were transferred by touching the loop to sterile drops. Similarly a loopful of parent medium without a paramecium was just touched to a second drop and a third loopful was thoroughly mixed with another drop. The effect of a thin film of oil on the plates was also determined. The improved loop and washing procedure previously described were used throughout. The paramecia were from a 13-day old culture of the strain used before.

TABLE III

EFFECTIVENESS OF LOOP TRANSFER METHOD IN REDUCING NUMBER OF BACTERIA TRANSFERRED IN THE CULTURE MEDIUM

Para- me- cium No.	Plates oiled							Cultures after 6 days at 20° C.	Plates not oiled				
	Number of bacteria per loop	Number of bacteria transferred by touching loop to drop	Wash No.						Wash No.				
			1	2	3	4	5		1	2	3	4	5
			Number of bacteria per wash						Number of bacteria per wash				
1	2000	600	500	2	21	3	1	Sterile	180	14	2	1	1
2	1400	900	800	1	15	0	0	Sterile	270	13	2	0	0
3	2900	1000	1000	5	7	0	0	Sterile	400	5	2	0	0
4	2600	1200	1500	16	3	0	0	Sterile	320	14	4	0	0
5	2400	1000	1300	3	1	2	0	Sterile	420	14	5	0	0
6	1900	1200	1300	14	5	0	0	Sterile	400	7	3	0	0
7	2600	1300	800	2	3	0	0	Sterile	480	55	6	1	0
8	2700	1600	1000	23	8	0	0	Sterile	280	0	6	0	0
9	2800	1000	900	9	5	0	0	Sterile	400	4	2	1	1
10	1700	1800	1600	5	3	0	0	Sterile	320	3	9	0	0
11	2900	500	1200	2	2	0	0	Sterile	250	8	3	0	0
12	1800	900	1500	1	3	1	0	Sterile	170	1	11	0	0
13	2000	1700	1100	7	0	0	0	Sterile	460	4	1	0	0
14	3000	1200	1900	5	5	0	0	Sterile	270	14	4	0	0
15	2100	500	1700	4	18	0	0	Sterile	250	4	2	0	0
16	1600	800	1500	8	9	0	0	Sterile	300	0	3	0	0
17	2500	1400	1600	14	0	0	0	Sterile	90	8	2	0	0
18	2100	700	1300	4	33	0	0	Sterile	440	2	2	0	0
Total	41,000	19,300	22,500	125	141	6	1		5700	170	69	3	2
Average	2278	1072	1250	7	8	0.3	0.05		317	9	4	0.2	0.1

NOTE: (a) Number of bacteria in parent culture: 26,500,000 per cc.

(b) Tube culture of 18 paramecia from second series after 6 days at 20° C. was sterile.

Results in Table III show that only about half as many bacteria were transferred by touching the loop to a sterile drop as by transferring the entire contents of the loop. The additional number of bacteria carried over by the

transfer of an animal was comparatively small and most of these were left behind in the first wash. The culture medium itself was the largest contributor of bacterial contamination. The count can be reduced by about 1/10,000 per transfer by the loop method, near sterility of the medium being reached at the second transfer. Once this is obtained it is only necessary to allow sufficient time in succeeding washes for the animals to free themselves from closely adhering or ingested bacteria. The indications are that by further adjustment of the washing period, freedom from bacteria may be reached in still fewer washings.

The bacterial numbers observed in the first washes from oiled and unoled plates show that about four times as many bacteria were transferred in the former. This indicates that more of the liquid medium was carried across when oil was present. Oil bubble reflections made it difficult to locate paramecia in the wash drops and to read the number of bacterial colonies on the plates. Oil had little effect on preventing the spread of drops.

Some Preliminary Growth Experiments

In the first experiment none of the paramecia lived for longer than seven days and no divisions occurred. It was found in a previous study that the majority of single isolates lived only a few days and failed to divide regardless of the bacterial content of the medium. Yet hay infusion flasks sowed with a few millilitres from a heavy paramecium culture invariably provided a new heavy culture in the course of five or six days. These results suggested that perhaps the medium lacked a growth promoting substance produced in sufficient quantity only when a large number of animals were present. To test this supposition paramecia were cultured in duplicate tubes of Pringsheim's medium containing 1 and 10 parts per million of the following substances: ascorbic acid, calcium pantothenate, nicotinic acid, oestriol, riboflavin, and inositol. Saturated and 1/10 saturated aqueous solutions of thyroxine were also used. In addition quadruplicate tubes were included combining all these substances in both the high and low concentrations. Since there was some indication that thyroxine might affect division, triplicate tubes of medium containing saturated, 1/4, 1/16, and 1/64 saturated aqueous solutions of thyroxine were also tried.

With the exception of two media (containing saturated and 1/10 saturated thyroxine) in which one division took place, no divisions occurred in any tube. No marked differences between treatments were noticed with the exception that 10 parts per million of riboflavin was harmful. The animals receiving these treatments and the higher concentration combination treatments died within one day. A few animals were dead each day. Ten of the 72 included in these tests were still living when the cultures were tested for bacteria on the sixth day.

Eighteen paramecia washed in the last series of experiments were put into one tube to determine if the association of a number of bacteria-free animals would have any effect on their reproduction. No divisions were observed and the death rate was similar to those placed in separate tubes.

Acknowledgment

The author wishes to acknowledge the assistance of Mr. A. M. Adams, Laboratory Assistant, National Research Laboratories.

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ON THE FOOD OF SEALS IN THE CANADIAN EASTERN ARCTIC¹

BY M. J. DUNBAR²

Abstract

The stomach contents of 47 ringed seals (*Phoca hispida*), five bearded seals (*Erignathus barbatus barbatus*), and one harp seal (*Phoca groenlandica*), from Baffin Island waters, are described.

It is shown that *Phoca hispida*, during the months of August and September at least, is predominantly a plankton-eater, the bulk of its food consisting of the pelagic amphipod *Themisto libellula*, and to a much lesser extent, the schizopod *Mysis oculata*. The stomach of the harp seal also contained planktonic species only.

The food habits of the ringed seal are discussed in relation to the plankton succession in the fjord waters.

Introduction

Stephensen (11) records the finding of large numbers of the planktonic euphausiid, *Thysanoessa inermis*, in the stomachs of harp seals (*Phoca groenlandica*), in east Greenland, and comments on the novelty of this occurrence: "As far as I know we have here for the first time euphausiids found in great multitudes in the stomachs of seal" and "Sometimes crustacea have been found in the stomachs of seals, but the records previously known were few, and the finds seem to be quite accidental." This is not strictly true. Andersson (1), Barrett-Hamilton (2), and Trouessart (13), all record that the Antarctic crab-eater seal feeds almost exclusively on planktonic crustacea, mainly *Euphausia superba*; this has also been noted recently by Bertram (3) and Lindsey (8). Barrett-Hamilton (2) found Weddell's seal, another Antarctic species, feeding on *Euphausia* in the pack ice, a fact that is not corroborated by other workers, though Lindsey (9) found the very young of this species feeding on *Euphausia*. Of the workers in the Arctic region, Johansen (6) found the stomachs of all the seals (*Phoca hispida*) shot in the drift ice during June and July full of pelagic amphipods, whereas Kumlien (7) and Hantzsch (5) found *Phoca hispida* feeding largely on crustacea of an undefined type. More recently Sutton and Hamilton (12) said of *Phoca hispida* taken in May at Southampton Island: "The stomachs we had opportunity to examine were in every case well filled with the remains of "Kingook", an abundant crustacean, and with small fish." (The Eskimo word "Kingook" is in point of fact applied to almost any small crustacean of an amphipod-like shape.)

The present paper describes material that shows that the bulk of the food of the jar seal, or ringed seal, *Phoca hispida* Schreber, in the waters of the Baffin Island coasts, consists of planktonic crustacea, at least in the summer months. Seals with such plankton-eating habits are apparently not found

¹ Manuscript received for publication February 19, 1941.

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in more temperate regions, where the plankton is neither so large nor so abundant. In view of the bad reputation of seals amongst fishermen, it is interesting to find how few seals are consistent fish-eaters.

Material

As a member of the Canadian eastern Arctic Patrols of 1939 and 1940, and while making a study of the marine plankton of the Baffin Island coasts, Hudson Strait, and northern Labrador, the author examined the stomachs of 47 ringed seals, five bearded seals (*Erignathus barbatus barbatus* Erxleben), and one harp seal (*Phoca groenlandica* Erxleben). The bearded seal is a strictly benthonic feeder, eating bottom-living crustacea, mollusca, and fish. The contents of the five stomachs examined consisted of the following:

Sclerocrangon boreas (Phipps)

Hippolyte (*Spirontocaris*) *polaris* (Sabine)

Hippolyte (*Spirontocaris*) *spinus* (Sowerby)

Remains of sculpin, probably *Myoxocephalus groenlandicus*
(Cuvier and Valenciennes)

One tubicolous worm, damaged and not identifiable.

The stomach of the harp seal, examined on August 8, 1940, at Frobisher Bay, was full of *Mysis oculata* (Fabr.) in large numbers, together with four

TABLE I

STOMACH CONTENTS OF *Phoca hispida*, 1939. SPECIES IN BOLD-FACE TYPE REPRESENT BULK OF CONTENTS

Date	Station	Number of seals	Stomach contents
Aug. 2	Lake Harbour	1	<div> <i>Themisto libellula</i> </div> <div> Full Full Five full, one half full Five full, five half full, one almost empty </div>
Aug. 4	Lake Harbour	2	
Aug. 4	Lake Harbour	6	
Aug. 7	Lake Harbour	11	
			Also, in these 20 stomachs:
			<i>Hyperia spinigera</i> 1 specimen
			<i>Pseudolibinia nanseni</i> 2
			<i>glacialis</i> 8
			<i>Gammarus locusta</i> 5
			sp. 1
			<i>Gammaracanthus loricatus</i> 1
			<i>Meganocypharus norvegica</i> 1
			<i>Thysanoessa raschii</i> 1
			<i>Mysis oculata</i> 14
			<i>Melchiorimysis mixta</i> 1
			Vertebral column and muscle of small fish not identifiable
Aug. 7	Lake Harbour	2	Empty
Aug. 7	Lake Harbour	1	Liquid only.

TABLE II

STOMACH CONTENTS OF *Phoca hispida*, 1940. SPECIES IN BOLD-FACE TYPE REPRESENT THE BULK OF THE CONTENTS

Date	Station	Number of seals	Stomach contents
Aug. 4	Gabriel Strait S.E. Baffin Is.	1	<i>Mysis oculata</i> Full <i>Themisto libellula</i> 4 specimens
Aug. 13	Lake Harbour	1	<i>Themisto libellula</i> Full
Aug. 13	Lake Harbour	1	Empty
Aug. 20	Lake Harbour	2	Empty
Aug. 20	Lake Harbour	5	<i>Themisto libellula</i> Full <i>Thysanoessa raschii</i> 1 specimen
Aug. 20	Lake Harbour	1	<i>Mysis oculata</i> Full <i>Themisto libellula</i> 2 specimens <i>Hippolyte ?gaimardi</i> 1
Aug. 22	Lake Harbour	4	<i>Themisto libellula</i> Full
Aug. 22	Lake Harbour	1	<i>Themisto libellula</i> Half full
Sept. 9	Clyde River N.E. Baffin Is.	2	Almost empty, contained altogether: <i>Themisto libellula</i> 1 specimen <i>Mysis oculata</i> 18 <i>Micthemysis mixta</i> 3 <i>?Myoxocephalus groenlandicus</i> 2
Sept. 14	Clyde River	1	
Sept. 17	Clyde River	1	
Sept. 13	Clyde River	1	Empty
Sept. 14	Clyde River	1	
Sept. 17	Clyde River	1	
Sept. 17	Clyde River	1	About one-third full; contained: <i>Calanus finmarchicus</i> 1 specimen <i>Metridia longa</i> 2 <i>Themisto libellula</i> 1 <i>Apherusa glacialis</i> 2 <i>Gammarus locusta</i> 5 <i>Thysanoessa inermis</i> 1 <i>Mysis oculata</i> 33 <i>Micthemysis mixta</i> 1 Decapod larvae 4 <i>Limacina helicina</i> 142 <i>?Cyclogaster fabricii</i> 1 Scraps of small fish, very little

specimens of *Mysis mixta* Lilljeborg, and 22 specimens of *Themisto libellula* (Mandt). *Mysis oculata*, although usually found near the bottom in shallow water, must be considered a planktonic species. It is caught in the tow-net rather than in the dredge.

The contents of the stomachs of *Phoca hispida* are given in Tables I and II. Species in bold-face type represent the greater proportion of the contents. The complete list of animals found in *Phoca hispida* is as follows:

Calanus finmarchicus (Gunnerus)

Metridia longa (Lubbock)

Hyperia spinigera Bovallius

Themisto libellula (Mandt)
Pseudalibrotus nanseni G. O. Sars
Pseudalibrotus glacialis G. O. Sars
Apherusa glacialis (H. J. Hansen)
Gammarus locusta (Linné)
Gammarus sp.
Gammaracanthus loricatus Lovén
Meganyctiphanes norvegica (M. Sars)
Thysanoessa raschii (M. Sars)
Thysanoessa inermis (Krøyer)
Mysis oculata (Fabr.)
Mysis mixta Lillj. (= *Mictheimysis mixta* (Lillj.))
Decapod larvae
Hippolyte (*Spirontocaris*) ?*gaimardi* Milne-Edwards
Limacina helicina (Phipps)
? *Cyclogaster fabricii* (Krøyer)
? *Myoxocephalus groenlandicus* (Cuvier and Valenciennes)

Results and Discussion

It is apparent from Tables I and II and from the species list that *Phoca hispida*, at least during the months of August and September, is predominantly a planktonic feeder in Baffin Island waters. The work of Kumlien (7), Sutton and Hamilton (12), and Johansen (6) extends the time-scale of this habit back to May, and includes Southampton Island and northeast Greenland in its geographical range.

There are other points of interest. The seals examined in August, 1939 and 1940, in southern Baffin Island (Lake Harbour and Frobisher Bay) were, with very few exceptions, well fed, and at that time there was an abundance of *Themisto libellula* in the plankton. Towards the latter half of August, and in September, *Themisto* was scarce in the plankton of the coastal water, both at Lake Harbour and in the north, at Clyde River, and the seals at Clyde River, taken in September, were virtually starving. It is apparently not possible for them to make up their diet with fish; nor, it seems, can they dig for bottom-living crustacea and mollusca as the larger *Erignathus* is able to do; other planktonic species are either not of sufficient size or in sufficient numbers to make up for the lack of *Themisto*. The result is that the ringed seal must go through a lean time in the late summer. Anything available is eaten—it is interesting that one seal was found eating the small planktonic mollusc *Limacina helicina*, a species that appears in enormous numbers at that time, following on the *Themisto* population.

The fate of the *Themisto* population is not relevant to this paper and will be discussed elsewhere; but with the disappearance of *Themisto* from the fjord waters during the summer months, there is an accompanying scarcity of seals. According to information from residents in the country, both White and Eskimo, they return late in September or in October, and it seems that in winter

and spring the ringed seal is still eating "small crustacea". (Kumlien (7), however, found them subsisting mainly on fish in Cumberland Gulf during the winter.) The occurrence of ringed seal, perhaps, follows the greatest abundance of the larger planktonic crustacea, notably *Themisto libellula* in the Baffin Island area, and it is possible that the starving seals examined at Clyde River were stragglers who failed to follow the majority.

There is, however, another possible explanation of the behaviour. The ringed seal is not simply an aquatic mammal; it is an animal whose habits are closely associated with the ice, and it seems more probable that the majority leave the fjord waters with the ice, in the early summer (July), and that those left behind are responsible for the drastic reduction of the numbers of *Themisto libellula* as shown by the results of the tow-netting operations in August. Once the *Themisto* population is depleted so as to be no longer a food supply, the seals left in the fjords become scavengers, mainly on the plankton.

In this connection, information is plainly needed concerning the following points:

1. The plankton populations in the neighbourhood of the ice in the open water of Hudson Strait and Baffin Bay.
2. The food habits of the seal in these open waters in August and September.
3. The nature of the plankton, and the food of the seals, at the time of freeze-up in the fall.

Two of the seals, those caught on August 4 and August 20, 1940, had been eating *Mysis oculata*, not *Themisto libellula*. One of these was from Gabriel Strait, and the tow-netting operations carried out there confirmed the inference from the stomach of the seal, that *Themisto* was scarce in the locality. The other came from near Lake Harbour, and was shot by an Eskimo not more than 20 miles from the settlement. Five other seals brought in on the same day had their stomachs full of *Themisto*, as did those examined on August 22, two days later (Table II). It may be inferred from this that the distribution of one of these species is patchy, according to which one is preferred by the seals; it seems to be *T. libellula*. In either case, whether the seal is eating *Mysis* or *Themisto*, the method of feeding is the same, i.e., planktonic.

There appears to be a significant difference in the manner of feeding of the two plankton-eating seals, *Phoca hispida* in the Arctic and *Lobodon carcinophagus*, the crab-eater seal, in the Antarctic, in that the former is selective and the latter is not. Barrett-Hamilton (2) quotes M. Racovitza on *Lobodon* as follows: "Il nage la bouche ouverte dans les bancs de ces crustacés, (*Euphausia superba*), à la façon des baleines, et en consomme de grandes quantités." And he adds: "I think it very probable that some light is thus thrown upon the wearing and use of the extraordinarily complicated cheek-teeth, the cusps of which may form a sieve through which is strained the water taken into the mouth with the euphausiids." No such behaviour of *Phoca hispida* has ever been recorded. Furthermore, during the summer in Baffin

Island waters the *Themisto* show a strongly bimodal size distribution, similar to, but more strongly marked than, the size distributions described for other Arctic planktonic animals from Greenland by Stephensen (10) and Dunbar (4). Only the larger-size group of *Themisto* was found in the seal stomachs, and it may be assumed that the ringed seal takes only the larger specimens. The dentition of *Phoca hispida* is not of the *Lobodon* type, and indeed it is remarkable that an animal that feeds so much on small crustacea should have such sharp and efficient teeth. The crustacea are almost intact, for the most part, when they are found in the stomach.

Parasitic worms were plentiful in the stomachs of the bearded seal, and also in the single specimen of the harp seal, but *Phoca hispida* was comparatively free of them. Seals examined in 1939 showed none at all, and in 1940 only three individuals possessed parasites, in small numbers. All three were taken at Clyde River, and were starving. Bertram (3) found, similarly, that the plankton-eating *Lobodon* of the Antarctic "rarely harboured a single species" of gut parasite, whereas the fish-eating Weddell's seal was full of them. It would seem that the parasites are derived from the food eaten.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

JUNE, 1941

NUMBER 6

CANADIAN WILTSHIRE BACON

XIX. COMPARATIVE FLAVOUR TESTS ON CANADIAN AND DANISH BACONS¹

By C. A. WINKLER² AND W. H. COOK³

Abstract

Tests of the comparative flavour quality of Danish and Canadian bacon, conducted in England by jury groups and a large body of representative consumers, showed, on the average, that Danish bacon was preferred. Nevertheless the product from certain Canadian factories was of quality comparable with the best Danish bacon. This shows that the better curing practices and transport conditions already developed in Canada, in use when these tests were made, are capable of delivering to the British consumer a generally satisfactory product. The average quality of Canadian bacon would therefore be improved by the uniform adoption of these improved practices. Although toughness, texture, flavour, and fat of poor quality may all have contributed to the inferiority of Canadian bacon, excessive saltiness appeared to be the principal complaint.

Introduction

The results of several physical, chemical, and bacteriological studies on Canadian Wiltshire bacon have been presented in recent communications from these laboratories (1-4, 6-13). Although palatability, particularly with respect to flavour, was recognized as the fundamental criterion of quality, no attempt was made to examine this factor for two main reasons. (a) Flavours can only be judged subjectively, and to take account of consumer preference it is obviously necessary to make flavour tests with representatives of the consuming public concerned. Since practically all the Wiltshire bacon of Canadian manufacture is exported to England a reliable consumer estimate of its palatability could not be obtained except by experiments in which representative English consumers acted as judges. (b) Flavour tests made in Canada could, at best, be arranged to take only doubtful cognizance of possible changes in flavour during transport and subsequent smoking and distribution.

To satisfy these two main requirements for reliable information, all the flavour tests reported in this paper were made in England under the supervision of a representative of the National Research Laboratories (C.A.W.).

¹ Manuscript received April 3, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 64 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 994.

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Experimental Designs

Approximately 50% of the bacon consumed in England at the time of these studies was of Danish origin. Whether as a result of acquired preference by the consumers, or of intrinsic superiority of the product, Danish bacon may be regarded as a standard of Wiltshire bacon quality on the English market. Hence, the investigations were primarily designed to compare the qualities of Danish and Canadian bacons as reflected in consumer preference, especially in relation to palatability. Experiments were also designed, however, to determine the variability in product exported from various factories in Canada.

Of the Canadian bacon received in England, approximately 75% is consumed in the southern counties, particularly around London, and the remainder in the northern counties. In the south, the bacon is retailed as a smoked product, whereas in the north the major portion is consumed in the pale condition. There is, moreover, a difference in selection and cut in the two areas. Investigations were therefore made in both areas.

A. London Area

The studies in the London area were of several types.

1. Jury Group Tests with Pale and Smoked Bacons

To obtain information about the effect of smoking, there was designed, according to a factorial scheme, an experiment in which smoked and pale bacons from 10 Danish and 10 Canadian factories were to be provided to two jury groups of six people each. The original design called for four samples of pale bacon, representing a random selection of factories, to be tested each day by one group, and four samples of smoked bacon from the same sides as the pale materials to be tested by the second group. Tests were to be made on each set of four samples on two successive days, so that in 10 days samples from all 20 factories would have been tested in duplicate. The whole experiment was then to have been repeated, but owing to difficulties in securing materials, it was not possible to reproduce exactly, in this second half of the experiment, the arrangement of samples used in the first half. In consequence of this the data were amenable only to limited statistical analyses, and are correspondingly limited in their significance.

2. Canteen Tests

Smoked material from 10 Danish and 10 Canadian factories, taken at random, was provided in sample pairs, representing all possible combinations of the 20 sides to be tested, to various canteens in the London district. Patrons of the canteens served as judges. In all, 360 sample pairs were provided, 18 sample pairs being furnished each day for 20 days.

3. Incomplete Randomized Block Tests

Samples from 252 sides of smoked bacon, representing the product from 10 Danish and 18 Canadian factories taken at random, were tested by two entirely independent jury groups of nine people each. The samples were

combined to conform to a 7×9 balanced incomplete randomized block scheme, and each jury member compared four samples per day during seven days. At the end of this time, each member had tasted all the samples once. This experiment had a decided advantage over the other experiments that were made, since in it nine instead of two sides were used to represent each factory, thus affording a reliable estimate of the palatability of the product from each factory.

4. *Family Group Tests*

Simultaneously with the incomplete randomized block scheme described above, sample pairs from the same materials, in all possible combinations of Danish and Canadian bacons, were provided to 54 households in London and the surrounding provinces. Each household received a sample pair each day during seven days.

5. *Consumer Tests*

To supplement the information from the jury group and family tests, and to satisfy the desirability of having a reasonably large cross section of the population represented, sample pairs of smoked materials from a random selection of 10 Danish and 10 Canadian plants, representing all possible combinations of the 20 samples, were provided to 1330 consumers in and around London. The experiment was repeated the following week. It was not possible to duplicate exactly the factories represented in the two tests.

6. *Tests in J. Lyons & Co. Laboratories*

A limited number of tests were made by experienced tasters in the laboratories of J. Lyons & Co., Hammersmith, with smoked material from 10 Danish and 10 Canadian factories. A jury group of six people was employed, the system of scoring being that to which they were accustomed and more detailed than that used in the other experiments. This point will be considered further in a later section.

B. *Northern Area*

A consumer test, similar to A5, was made in the northern area (Liverpool, Manchester, Birmingham, Hull, Leeds). Pale bacon was used, however, and the method of cutting commonly employed in this area was adopted. Sample pairs representing all combinations of Danish and Canadian bacons from a random selection of 10 factories of each country were provided to 1140 consumers for comparison. The experiment was repeated the following week. The same Canadian, but not the same Danish factories were represented in the two experiments.

Experimental Materials and Methods

Although it would have been preferable to have taken the experimental materials from retail shops, such a procedure was impractical due to the great difficulty, if not impossibility, of ensuring that the desired materials could of certainty be obtained. The bacons were therefore obtained directly

from the wholesale distributors. The materials for Experiments *A1*, *A2*, and *A5* were smoked by one wholesale firm, those for *A3*, *A4*, and *A6* by another firm. The factories included in any given experiment were representative of those exporting regularly at the time.

In Experiments *A1* to *A4* and Experiment *A6*, 2 to 3 lb. portions of the long back immediately behind the ribs were removed for experimental materials. In Experiment *A5*, the whole of the long back was used, excluding material from within 2 to 3 in. of the salt pocket to the collar. For experiments in the northern area, the whole of the so-called "middle" from each side was used; this middle represents the bacon side from which the fore-ends and gammons are squarely cut, and includes all the streak, whereas only a small amount of streak remained on the long back used in the London area. These conditions represent accepted practice in the respective markets.

Bones were removed from all materials and samples sliced to uniform thickness on a machine. The slices, properly identified and allocated according to the experimental design, were immediately wrapped in grease-proof paper for future dispensation.

With sample pairs, the presence or absence of rind afforded the means by which the tasters identified the samples submitted to them for comparison. In each case, a record available only to the supervisor enabled subsequent identification for compilation of data. In the jury group tests, the samples were identified by their positions on the laboratory bench.

Samples for the jury groups were kept in a refrigerated room at 32° F. from the time of sampling until the time of cooking. This storage period was seldom more than 15 hr. Samples for household tests were sent through the mails, well wrapped and supported between pieces of cardboard. Dispatch was made immediately after sampling in late afternoon or evening and the samples were generally available for testing at breakfast the following morning. An instruction sheet and a score card were included in each envelope. Samples for the canteen tests and the consumer tests were delivered personally within 24 hr. of sampling, being held in a refrigerated room until time of delivery.

All samples tested by the jury groups were grilled, four samples being cooked at one time. Since the fat dripped through the grill no mingling of flavours was possible, unless it occurred by absorption of volatile substances. This doubtless was negligible. All the cooking for each jury group was done by a cook assigned to the group. The samples for one group were prepared on an electric grill, those for the other group on a gas-fired grill. Care was taken to cook the samples to about the same degree of crispness.

Cooking in the family group and large consumer tests could not, of course, be supervised. However, an instruction sheet accompanying each sample pair emphasized the necessity for cooking each sample separately in a clean frying pan. The extent to which this procedure was followed is not known,

but there is reasonable assurance that, at least in the smaller test involving 54 households, the instructions were well obeyed.

Cooking for the canteen tests was done by the staff in charge of cooking operations in the particular canteen. For the most part, these samples were grilled.

The tests carried out by Lyons & Co. were made on samples grilled by an experienced laboratory technician.

Where selection of personnel was possible, only persons thoroughly accustomed to eating Wiltshire-cured bacon were chosen. Selection was not possible, of course, in the canteen and large consumer tests.

One jury group, operating at the Smithfield Laboratory of the Food Investigation Board, Department of Scientific and Industrial Research, and designated in future as the Smithfield jury group, was composed of the officer-in-charge of the laboratory, his assistant, a university student, the proprietress of a restaurant, three members of an office staff, one of whom was a woman, the superintendent of a building, and a male employee of some 40 years' experience with a firm of bacon importers.

A second jury group, operating at the firm of Sheed-Thomson & Co., and designated hereafter as Importers' jury group, was composed entirely of members of the office staffs of firms importing bacon into England.

Little information about the personnel of the 54-family group was available. The group comprised several members of a university staff and various executives of business firms, and the more immediate friends of these two classes.

The personnel of the 12 canteen test groups for which data are available represented a variety of occupations and salary ranges. Included were the clerking staffs, assistant buyers and buyers of departmental stores, waiters, assistant chefs and chefs of hotels, the clerking staff of a social organization, assistant grocers, factory hands, and the staff of a cheese importing firm. There were both male and female tasters whose salary ranges could be distinguished as low, medium, and high. Although in some groups all salary ranges prevailed, in other groups low, low to medium, or medium to high ranges defined the limits.

The large consumer tests in both London and northern areas also represented a wide salary range, among employees of several firms through which the samples were distributed.

The jury groups compared four samples at a sitting, each member working quite independently of other members of the group. No effort was made to compel a taster to adopt any of the frequently recommended procedures such as rinsing the mouth with water, or eating a piece of apple or bread between tasting different samples. Occasionally, a water rinse was voluntarily used, particularly if a sample was distastefully salty. The majority of the tasters preferred to swallow the sample after chewing it, and this was not discouraged.

It was felt that each taster should be allowed to function in the manner considered most desirable from his or her personal point of view.

In the family group tests, the large consumer tests, and the canteen tests, no restrictions whatever were placed on the method of tasting, even to the point of permitting other foods to be eaten with the bacon in the normal way if desired. No doubt academic objections might be raised to these methods, but from a practical point of view there is probably much to commend them.

In all tests except those conducted by Lyons & Co., the taster was simply required to place the samples provided in order of preference stating, where possible, reasons for the preference.

The jury groups were required to place four samples in order of preference at each sitting; the consumer tests, family, and canteen tests involved only a distinction between one sample pair per sitting. The taster was free to state, where desired, that no distinction was possible between any two or more samples provided.

On the basis of the preference so obtained it is possible to assign numerical values. For sample pairs, these are simply +1 for the preferred sample, -1 for the sample not preferred and 0 for each if neither sample is preferred. With four comparisons the scores are 1.03, 0.30, -0.30, and -1.03 in decreasing order of preference (5). The intervals between successive scores were chosen to transfer the ordinal numbers obtained in scoring to normal deviates amenable to analysis of variance. If any two or more samples are indistinguishable, each is assigned the average of the scores corresponding to the two or more positions taken by the identical samples in the order of preference.

Results

1. *Jury Group Tests with Pale and Smoked Bacons (London Area)*

As mentioned previously, the original design of these experiments was factorial. Owing to circumstances over which no control could be exercised, however, the second half of the experiment could not be made a duplicate of the first half, nor could the experiments of the last day be completed.

Since samples from the same factories were tested on two successive days, comparisons between days are possible but, since the grouping of the factories within days was not the same for the duplicate tests as during the first 10 days' experiments, comparisons between sides are not possible. The procedure adopted was to treat the results from the second half of the experiment as if an additional number of factories was represented, rather than duplicate tests of material from the same factories represented in the first half of the experiment. Also, since results for the last day could not be obtained, those for the second last day were omitted. A total of 72 tests, obtained during 18 days, by each of the two jury groups remains for consideration.

Since comparisons are possible within each two-day period, the results from nine groups of four factories each may be treated independently, and the observed variance allocated as shown in Table I. The difference between

TABLE I

ANALYSIS OF VARIANCE OF JURY GROUP DATA WITHIN SUCCESSIVE TWO-DAY TEST PERIODS
FOR FIRST 18 DAYS OF TEST

Variance due to:	D.f.	Mean square	
		Smithfield jury, smoked bacon	Importers' jury, unsmoked bacon
Between factories (within two-day test periods)	27	129**	89.1*
Between days (factories \times days)	27	23 0*	44 0**
Residual	270	16.0	19.0

Bacon	Total score	Bacon	Total score
Pale:		Smoked:	
Danish	265 35	Danish	373 08
Canadian	-265 35	Canadian	-373 08

* Indicates 5% level of significance.

** Indicates 1% level of significance.

NOTE: Significance of differences between factories determined by comparison with variance between days and latter compared with residual.

days, which is the interaction of factories \times days, significantly exceeds the residual variance for both jury groups, and the difference between factories has therefore to be tested against this interaction. On this basis, the difference between factories is found to be significant within two-day test periods, averaged for all days for both jury groups.

If the scores are totalled over the daily periods, 14 in all, in which two Danish and two Canadian sides were tested on each day, comparable scores by countries are obtained and these are reported in the last part of Table I.

Evidently, Danish bacon showed a superiority over Canadian in both the pale and smoked conditions. The figures also indicate that flavour quality in the pale and smoked conditions are directly related, and that inferiority in the pale condition is exaggerated by smoking. However, these indications are open to question in the absence of an estimate of the difference between sides from the same factory, against which the differences recorded above must be tested for significance.

2. Canteen Tests (London Area)

The results for the canteen tests are even more limited than those described above for the jury group tests on the same materials owing to commencement of hostilities in Europe, which disorganized arrangements for the return of the score cards distributed with the samples. Information for days 13 and 15 to 20 inclusive was lacking, and the results for the first 12 days only are therefore considered. Owing to the method of scoring, by preference

only, and the fact that different canteens were used each day, only a limited statistical treatment is possible.

Since two canteens tested the same samples on successive days, it is possible to analyse the variance into portions attributable to within and between canteens. The results of such an analysis are shown in Table II. Not only is the difference between canteens not significant, but the mean squares are almost identical. This demonstrates the rather interesting point that although some variation may occur between different individuals, ability to discriminate between bacon flavours did not seem to bear any relation to salary class or occupation.

TABLE II
ANALYSIS OF VARIANCE OF RESULTS OBTAINED IN THE
CANTEEN TESTS

Variance due to:	D.f.	Mean square
Between canteens	36	0.85
Within canteens	144	0.81

In Table III are given the total mean scores, arranged by test periods and country of origin of the product. The results are comparable only within test periods but the totals by countries over 10 test periods confirm the results of the jury group tests in showing the Danish product to be superior.

TABLE III
TOTAL MEAN SCORES FOR SMOKED BACON FROM FACTORIES TESTED IN CANTEEN EXPERIMENTS

Tests (days)	Canadian factories	Total mean scores × 100	Danish factories	Total mean scores × 100
1-2	C-1	00.0	D-1	66.0
	C-2	-82.0	D-2	16.0
3-4	C-3	00.0	D-3	00.0
	C-4	-50.0	D-4	50.0
5-6	C-5	00.0	D-5	-16.0
	C-6	-84.0	D-6	100.0
7-8	C-7	84.0	D-7	00.0
	C-8	-100.0	D-8	16.0
9-10	C-9	50.0	D-9	-66.0
	C-10	-16.0	D-10	32.0
	Totals	-198.0	Totals	198.0
11-12	—	—	D-4	00.0
			D-6	-50.0
			D-1	66.0
			D-2	-16.0

NOTE: Results comparable within tests (days) only. Necessary difference within tests = 103.

3 and 4. Incomplete Randomized Block and Family Group Experiments (London Area)

The samples used for the jury group tests according to the randomized block scheme were taken from the same sides as the sample pairs for the family group tests, and it is convenient to examine the results of these two experiments together. Complete analyses of variance were possible on these data, and little comment is necessary.

In Table IV, the factories have been arranged in decreasing order of total score over both jury groups for each country of origin. The standard error

TABLE IV
FACTORY TOTALS OBTAINED IN JURY GROUP AND FAMILY GROUP TESTS

Factories (in order of total score for both jury groups)	Group A (Smithfield)	Group B (Importers')	Sum (A + B)	Difference (A - B)	Family group
Canadian ¹					
A-1	265	59	324	206	7
B	142	4	146	138	7
A-2	18	90	108	-72	3
C-1	156	-76	80	232	-5
D	-21	58	37	-79	-1
E	-103	139	36	-242	-5
A-3	98	-106	-8	203	4
F	37	-64	-27	102	-6
G-1	-106	62	-44	-167	-4
H	-236	184	-51	-420	1
C-2	-166	98	-68	-264	-10
C-3	-243	165	-79	-408	-1
I	-100	-106	-206	5	1
G-2	-180	-118	-299	-62	-8
G-3	-80	-260	-340	180	-1
J	-287	-120	-407	-167	-3
K	-164	-314	-478	151	-9
L	-282	-207	-489	-75	-4
Danish ¹					
M	349	120	469	229	4
N-1	251	149	401	102	5
O	269	106	375	164	5
P-1	160	120	279	40	12
Q-1	152	18	170	134	4
Q-2	-134	295	161	-429	2
P-2	8	49	57	-41	9
R-1	100	-82	18	183	5
R-2	37	-40	-3	77	-4
N-2	59	-221	-162	281	-8
Standard error of single plant total	±163	±169	±235	±235	±7.81
Necessary difference, 5% level of significance, between any two factory totals	452	468	651	651	14.69

¹ Letters and associated numbers designate companies within nations, and factories within companies, respectively.

of a single factory total, and the differences necessary for statistical significance between two such totals, are recorded at the bottom of the table.

The corresponding data for the total scores over the two jury groups, and for their differences, are also given, together with the data for the family group tests.

Tables V and VI give the results of variance analyses on the data of Table IV. From Table V it is evident that the Smithfield Laboratory jury group

TABLE V

ANALYSES OF VARIANCE OF FACTORY TOTALS OBTAINED IN JURY GROUP TESTS

Source of variance:	Group A (Smithfield)		Group B (Importers')		Sum (A + B)		Difference (A - B)	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Between all factories	27	458,482**	27	311,014	27	456,729**	27	312,767
Canadian vs. Danish	1	3,480,005**	1	583,453	1	3,456,656**	1	603,868
Between Canadian	17	370,487	17	305,909	17	365,790	17	310,778
Between Danish	9	288,971	9	290,387	9	295,178	9	284,180
Error	162	230,015	162	247,534	324	238,774	324	238,774
Canadian								
Between companies	11	407,841	11	245,438	11	484,817*	11	259,371
Between factories within companies	6	307,005	6	416,772	6	147,448	6	405,024
Danish								
Between companies	5	189,141	5	206,938	5	269,373	5	250,019
Between factories within companies	4	416,944	4	394,647	4	327,435	4	326,882

* Indicates 5% level of significance.

** Indicates 1% level of significance.

TABLE VI

ANALYSES OF VARIANCE OF FACTORY TOTALS OBTAINED IN FAMILY GROUP TESTS

Source of variance:	D.f.	Mean square
Between all factories	27	2.42**
Canadian vs. Danish.....	1	12.9**
Between Canadian.....	17	1.83
Between Danish.....	9	2.38*
Error	351	1.17
Canadian		
Between companies	11	2.35*
Between factories within companies	6	0.88
Danish		
Between companies	5	1.41
Between factories within companies	4	3.60*

* Indicates 5% level of significance.

** Indicates 1% level of significance.

was able to demonstrate a significant difference between factories, taken over all factories regardless of origin, and a highly significant difference between Danish and Canadian bacons. The data from the Importers' group, although subject to only a slightly larger mean square error, did not demonstrate any source of variance to be statistically significant. However, the results based on the totals over both jury groups show the differences, not only between factories and between Danish and Canadian bacons, but also the difference between Canadian companies, to be significant. Hence, the individual results of the two groups must be supplementary.

An analysis of variance of the difference between the two jury groups shows none of the sources of variance recognized in the tables to be statistically significant. Since this analysis really represents the different reactions of the two groups to the quantities reported in the table, it is evident that one group did not prefer one, and the second group another type of bacon.

Variance analyses on the results of the family group test, Table VI, give support to the conclusions reached from the jury group tests. Significant differences between factories, taken over all factories, and between Danish and Canadian bacons were again demonstrated. Three other sources of variance were, however, shown to be significant, namely, between Danish factories over all Danish factories, between Canadian companies, and between Danish factories, within companies. For Canadian bacon, therefore, the major source of variance lies between companies, whereas for Danish bacon it lies within companies. The inference would seem to be that, whereas differences in flavour in Canadian bacons from different factories arise primarily from differences in curing and handling practices, variations in flavour of Danish bacons are presumably due to variations between pigs, or possibly to slight time to time variations in handling practices in a given factory.

Table VII summarizes the comments of the members of the jury groups. In summarizing these comments, only two classes were distinguished, namely, favourable and unfavourable. Unfavourable comments include those in which a sample was said to be very salty, salty, or slightly salty; if no comment was made on the saltiness, a favourable rating for this quality factor was assigned to the sample. If no comment whatever was made on a sample no rating was assigned, whereas if, for example, a taster reported the sample as both tough and salty, a double entry was made. All the attributes of quality mentioned by the tasters are included in the table. Since there were 18 Canadian factories and only 10 Danish factories, the summary is expressed on a percentage basis.

The comments summarized in this form show that, taken over all the recognized attributes of quality, both jury groups agreed that Canadian bacon is inferior to Danish, a fact that merely bears out the flavour scores. The remarks indicate that both Danish and Canadian bacons were considered too salty for the English palate, with no marked inferiority of the Canadian product in this respect; on the other hand, Canadian bacon suffered considerably more adverse criticism for toughness than did Danish bacon. A

TABLE VII

SUMMARY OF COMMENTS MADE BY JURY GROUP PANELS (SMITHFIELD AND IMPORTERS¹)

Quality factors	Favourable ¹		Unfavourable ¹		Unfavourable, as % of total	
	Group A (Smithfield)	Group B (Importers')	Group A	Group B	Group A	Group B
Canadian factories (18)						
Lean						
Saltiness	4	2	63	77	94.0	97.5
Toughness	18	20	15	29	45.5	59.2
Texture	3	19	4	32	57.2	62.8
Flavour	22	73	30	18	57.7	19.8
Miscellaneous	14	26	14	1	50.0	37.5
Fat	17	2	5	10	22.7	83.2
Total	78	142	131	167	63.9	54.1
Danish factories (10)						
Lean						
Saltiness	2	3	22	26	91.8	89.7
Toughness	12	17	5	7	29.4	29.2
Texture	0	11	1	12	—	51.1
Flavour	18	40	10	17	35.7	29.8
Miscellaneous	10	16	1	0	9.1	—
Fat	7	1	2	5	22.2	83.2
Total	50	88	41	67	45.0	43.2

¹ The ratings are based on comments for individual samples compared in blocks of four samples per test.

general conclusion regarding flavour, considered as a quality factor in itself, is scarcely possible, and the same is true for texture. The fats of the two types were more frequently criticized for off-flavour by the Importers' group, but there is little to choose between the fats as determining quality in the two bacons.

It is a point of considerable interest and importance that although Canadian bacon as a whole was regarded as inferior to Danish, the total scores recorded in Table IV show that it is not impossible for Canadian factories to turn out a product closely approaching the best Danish product in quality. A major problem remains, however, in bringing the quality of Canadian bacon generally to a higher level.

5. Consumer Tests (London and Northern Areas)

Although, as mentioned previously, 2660 sample pairs were distributed in the London area, and 2240 pairs in the northern counties, approximately 50% of the people in the tests failed to submit an opinion about the samples. Normally, the organization of the experiments would have been adequate to ensure returns in the neighbourhood of at least 90%, but international crises, culminating in war, made it impossible to supervise return of the score cards.

Owing to the incomplete returns, statistical analysis of the data is necessarily limited. The experimental error, pooled over both series, was computed for each of the London and northern areas, from the available number of degrees of freedom. The necessary difference for a given comparison was computed from these values. Since the score from each factory in a given series is represented by a *total*, the necessary difference appropriate to such a score is given by:

$$(\text{Mean square for individual comparison} \times n)^{\frac{1}{2}} \times t$$

The necessary difference therefore increases with the number of comparisons available for each factory, and since the number of returns per factory varies,

TABLE VIII
CONSUMER TESTS OF SMOKED BACON IN LONDON AREA

Factories (in order of score over both series)	Series I			Series II			Mean score for both series
	Number of factories in com- parison	Number of com- parisons between pairs	Total ¹ score	Number of factories in com- parison	Number of com- parisons between pairs	Total ² score	
Canadian 10 each series							
C-A1	19	58	- 1	14	41	15	7.0
C-B1	18	65	12	11	26	0	6.0
C-C1	18	73	15 ¹	14	50	- 9 ¹	3.0
C-D1	17	62	- 3	15	43	0	- 1.5
C-A2	19	66	-15	19	75	4	- 5.5
C-F1	—	—	—	18	54	- 7	- 7.0
C-F1	—	—	—	16	50	- 9	- 9.0
C-B2	19	72	-10	14	50	- 9	- 9.5
C-G1	—	—	—	16	58	-10	10.0
C-H1	19	77	-11	—	—	00	-11.0
C-I1	19	74	-22 ¹	15	40	-10 ¹	-16.0
C-12	19	77	-21	—	—	—	-21.0
C-13	19	76	-23	—	—	—	-23.0
Danish 10 each series							
D-1	19	70	17	—	—	—	17.0
D-2	19	66	15	—	—	—	15.0
D-3	—	—	—	18	45	13	13.0
D-4	18	78	16	16	41	2	9.0
D-5	—	—	—	14	34	8	8.0
D-6	18	62	3	16	44	9	6.0
D-7	19	63	16 ¹	15	46	- 6 ¹	5.0
D-8	18	75	5	14	44	5	5.0
D-9	18	65	5	—	—	—	5.0
D-10	—	—	—	13	39	4	4.0
D-11	—	—	—	16	52	3	3.0
D-12	18	59	5	16	48	0	2.0
D-13	18	67	2	14	50	- 3	- 0.5
D-14	19	79	- 5	—	—	—	- 5.0

¹ Factories in which there was significant difference between series.

² Within countries and series, the following differences in score, or greater, are statistically significant between plants:

Canadian, Series I: 24.
Danish, Series I: 17.

Canadian, Series II: 19.
Danish, Series II: 20.

the necessary difference for the score is different for almost every comparison that might be desired.

In the London area (Table VIII), individual comparisons between series, within factories, show that three factories differed significantly between series. In the northern area (Table IX), no comparisons for the Danish factories are possible, owing to the impossibility of having the same factories represented

TABLE IX
CONSUMER TESTS OF PALE BACON IN NORTHERN AREA

Factories (in order of score over both series)	Series I			Series II			Mean score for both series
	Number of factories in com- parison	Number of com- parisons between pairs	Total score	Number of factories in com- parison	Number of com- parisons between pairs	Total score	
Canadian 10 each series							
C-A1	18	51	1 ¹	19	64	24 ¹	12.5
C-B1	18	64	9	15	59	4	6.5
C-C1	17	56	15 ¹	16	64	-17 ¹	- 1.0
C-D1	19	73	- 2	16	64	0	- 1.0
C-E1	17	38	- 2	16	64	- 1	- 1.5
C-F1	15	47	1	11	64	- 6	- 2.5
C-G1	19	70	- 4	15	68	- 4	- 4.0
C-D2	19	68	- 7	14	58	- 2	- 4.5
C-B2	18	60	2 ¹	16	65	-17 ¹	- 7.5
C-E2	19	73	- 4	12	48	-17	-10.5
Danish 10 each series							
D-1	17	63	17	D-11	17	71	10
D-2	16	58	7	D-12	16	58	15
D-3	19	68	- 2	D-13	15	61	9
D-4	18	68	0	D-14	13	47	7
D-5	18	65	- 1	D-15	15	57	5
D-6	18	64	11	D-16	17	70	- 7
D-7	19	69	-11	D-17	13	53	3
D-8	15	49	-11	D-18	14	61	3
D-9	19	69	3	D-19	14	51	-11
D-10	18	65	-22	D-20	18	67	2

¹ Indicates factories showing significant difference between series within countries and series; differences in score of 19 or greater indicate statistically significant differences between factories.

in the two series. The results from three Canadian factories, however, showed significant differences between the two series. Several of the insignificant differences are large enough to suggest that, had the regular analysis of variance been possible, the difference between sides within factories (between series) would have been a significant source of variance. It appears from this that the results from two sides are not representative of the product from a given factory. If this is so, more complete returns probably would not have enabled more definite conclusions. The experimental error likely would have remained the same, but a significant difference between sides within factories

would probably have been the basis for comparing the differences between factories.

In view of the possible significant difference between series (between sides from the same factories), but lacking a suitable test of significance, each series has been treated separately. The difference between factories within series is therefore compared with the necessary difference arising from experimental error. Again, each pair of factories has a unique necessary difference, depending on the total number of tests for which returns were made. Such comparisons, although valid for the particular sides tested, may not be valid with respect to factories, owing to the limited number of sides used to represent each establishment. For this reason, and also to simplify and reduce the results, only the largest necessary difference for comparisons within a given nation and series are reported at the foot of each table.

The results in Table VIII show that even on this basis significant differences do exist between factories.

In Table X the total scores are given by countries and series, together with the necessary differences for determining whether they are significantly above or below the average (zero) and whether the total differences between scores are significant. The table is practically self-explanatory. In three out of the four tests, the difference between the scores for Danish and Canadian bacons are statistically significant. In one test (Series I, London area) Danish bacon was found significantly better than average.

TABLE X
CONSUMER TESTS OF PALE AND SMOKED CANADIAN AND DANISH BACONS

Area	Series	Scores			Necessary difference		
		Total for Canadian	Total for Danish	Total	From average (0) Canadian	From average (0) Danish	Between totals for countries
Smoked							
(London) L1	1	-79	79	0	44.8	44.3	71.2
(London) L2	2	-35	35	0	37.1	35.5	51.3
Pale							
(Northern) N1	1	9	9	0	39.3	40.5	56.4
(Northern) N2	2	-36	36	0	40.4	39.6	56.6

The fact deserves special comment that, whereas in the London area both series show a significant superiority of Danish over Canadian bacon, the tests in the northern area showed Danish superior to Canadian bacon in only the second series. The difference in the results from the two areas might be explained by the generally accepted belief that the people in the northern counties are less discriminating than those in the London area. However, it should be borne in mind that, whereas in the London area both types of

bacon are smoked shortly after arrival in England, similar measures of a preservative nature are not practised in the northern area. The apparently smaller superiority of Danish over Canadian bacon in the northern tests might therefore reflect a better keeping quality of the Canadian product in the pale condition.

Tables XI and XII summarize the available comments of tasters in the London and northern areas respectively. Only about 50% of the tasters commented on the samples, so that the numbers appearing in these tables do not correspond with those in the previous tables.

TABLE XI

SUMMARY OF COMMENTS MADE BY TASTERS ON SMOKED BACON; CONSUMER TESTS, LONDON AREA, SERIES I AND II

Quality factor	Favourable ¹	Unfavourable ¹	Unfavourable, as % of total
Canadian factories			
Saltiness	33	31	48
Toughness	10	12	55
Flavour (unspecified)	187	241	56
Miscellaneous	15	18	55
Total	245	302	55
Danish factories			
Saltiness	35	37	51
Toughness	14	12	46
Flavour (unspecified)	222	168	35
Miscellaneous	21	18	46
Total	292	235	45

¹ Ratings based on comments from 537 comparisons out of 1157 sets of paired samples on which flavour scores were obtained.

TABLE XII

SUMMARY OF COMMENTS MADE BY TASTERS ON PALE BACON; CONSUMER TESTS, NORTHERN AREA, SERIES I AND II

Quality factor	Favourable ¹	Unfavourable ¹	Unfavourable, as % of total
Canadian factories (10)			
Saltiness	56	54	49
Toughness	6	7	54
Flavour (unspecified)	205	226	52
Miscellaneous	61	70	53
Total	328	357	52
Danish factories (10)			
Saltiness	52	54	51
Toughness	10	9	47
Flavour (unspecified)	232	211	48
Miscellaneous	80	71	47
Total	374	345	48

¹ Ratings based on comments from 702 comparisons out of 1226 sets of paired samples on which flavour scores were obtained.

From the comments, the attributes of quality listed in the tables were recognized. The miscellaneous comments include such factors as colour, amount of fat, etc. Where the product received a favourable comment it was given a score of 1 and the sample with which it was in comparison a score of -1. The number of unfavourable comments is expressed as a percentage of the total, to take into account the variation in number of comments involving each type of bacon.

On the whole, the rating given by the comments reflects essentially the same results as the flavour scores, namely that superior samples were more frequently obtained from Danish bacon. There is no evidence that Canadian bacon was considered more salty than the Danish product, nor, apparently, was the toughness of Canadian bacon detrimental to an appreciable extent. In the unspecified flavour comments, however, smoked Danish bacon assumes a considerable margin of superiority in the London area, though this is not true for the tests in the northern area.

6. *Tests in J. Lyons & Co. Laboratories (London Area)*

Although very limited in scope, the tests made in the laboratories of J. Lyons & Co. are of considerable interest in view of the detailed system of scoring employed. Numerical values were assigned to each distinguishable characteristic of both lean and fat. For the lean, these characteristics are colour, taste, tenderness, juiciness, and flavour; for the fat, only taste, flavour, and texture were evaluated. Such a system of scoring has obvious advantages over the simpler preference rating used in the experiments already outlined, but if reliable results are to be obtained, requires operators, such as those in the Lyons' group, who are experienced in its use.

To obtain the data from the Lyons' group in a form suitable for statistical treatment, certain of the results had to be omitted. One member of the group was frequently absent, and the results from this individual were discarded completely, leaving five instead of six members represented. Results for one day had also to be disregarded, owing to substitution for one of the jury members. The results finally represent comparisons between eight Danish and nine Canadian factories.

The mean scores for each attribute for each factory and country are shown in Table XIII, arranged in decreasing order of total score. Necessary differences applicable to the means by factories and countries have been calculated and appear at the foot of the table. For certain attributes of quality some factories appear to differ significantly when compared on the basis of these necessary differences. However, an analysis of variance shows that for some of these attributes no significant difference exists in fact. In such cases the values have been put in parentheses. The apparent conflict arises from the fact that a 5% point computed from differences selected at random, is not the maximum difference to be expected when a number of comparisons are made. In these circumstances the variance analysis is the correct basis for assessing significance.

TABLE XIII
MEAN SCORES FOR EACH ATTRIBUTE OF QUALITY
FLAVOUR TEST DATA, LYONS' JURY GROUP

Factories and countries	Total over all attributes of quality	Scores							
		Lean					Fat		
		Colour	Taste	Tenderness	Juiciness	Flavour	Flavour	Taste	Texture
Possible score	100	15	10	15	10	15	15	10	10
Canadian									
C-1	84.0	13.6	8.3	13.2	8.5	12.2	11.9	7.9	8.4
C-2	83.4	12.2	7.6	12.7	8.1	11.7	13.7	8.8	8.6
C-3	82.0	12.1	8.4	11.5	7.6	12.0	13.0	8.8	8.6
C-4	80.6	12.8	7.3	12.2	7.7	10.4	13.5	8.5	8.2
C-5	80.0	11.6	8.2	11.8	7.5	11.4	12.6	8.7	8.2
C-6	79.9	11.8	7.5	12.0	7.5	11.8	12.5	8.6	8.2
C-7	78.3	12.9	7.6	10.9	8.1	10.4	11.9	8.5	8.0
C-8	75.2	11.0	6.7	11.5	7.5	10.7	12.4	7.9	7.5
C-9	74.7	9.7	6.6	11.9	7.9	11.0	11.9	8.1	7.6
Mean	79.79	11.97	7.58	11.97	7.82	11.29	12.60	8.42	8.14
Danish									
D-1	87.9	13.2	8.2	13.3	8.5	12.5	14.0	9.1	9.1
D-2	86.0	12.1	8.8	12.9	8.1	12.7	13.5	9.0	8.9
D-3	85.5	12.1	8.4	13.3	8.4	12.3	13.3	9.1	8.6
D-4	83.5	11.7	8.3	13.8	8.5	13.0	12.3	7.9	8.0
D-5	83.4	13.3	7.6	12.2	8.1	11.4	13.3	8.8	8.7
D-6	82.5	11.7	8.5	12.8	8.0	11.1	12.9	9.0	8.5
D-7	81.9	11.0	8.0	13.6	8.1	11.8	13.0	8.3	8.1
D-8	80.9	13.0	7.7	12.5	8.2	11.0	11.7	8.4	8.4
Mean	83.95	12.26	8.19	13.05	8.24	11.98	13.00	8.70	8.54
Necessary difference									
Between countries	1.90	(0.75)	(0.81)	0.68	0.34	0.47	(0.46)	(0.34)	0.24
Between factories	5.36	(2.20)	(2.85)	(1.98)	(0.98)	1.37	(1.34)	(1.00)	0.71

NOTE: Values in parentheses failed to reach level of statistical significance.

TABLE XIV
RESULTS OF ANALYSES OF VARIANCE ON THE FLAVOUR TEST DATA OBTAINED BY THE
LYONS' JURY GROUP

Source of variance	D.f.	Mean square								
		Total of all attributes of quality	Lean				Fat			
			Colour	Taste	Tender-ness	Juici-ness	Flavour	Flavour	Taste	Texture
Between all factories	16	123**	9.81	3.82	6.74	1.25	6.14*	5.00*	1.74	1.78*
Canadian vs. Danish	1	733**	3.70	15.7	49.7**	7.30*	19.9**	6.78	3.26	6.54**
Between Canadian	8	107*	13.8	4.25	4.65	1.25	4.74	4.63	1.32	1.53*
Between Danish	7	54.7	6.83	1.64	3.00	0.40	5.76	5.17	2.00	1.40
Error (factoriesXdays)	16	34.0	5.37	6.14	3.05	0.85	3.04	1.54	0.93	0.38

* Indicates 5% level of significance.

** Indicates 1% level of significance.

The main interest in the present investigation is the comparison of different factories and countries of origin. A detailed analysis of variance established the fact that the interaction "factories \times days" was an adequate estimate of error against which to test the significance of differences between countries of origin and between factories. The variance analysis given in Table XIV shows that for the totals of all attributes of quality the differences are significant between factories, over all factories, between Canadian and Danish bacon, and between Canadian factories, but are not significant between Danish factories. Tenderness, juiciness, and flavour of the lean and texture of the fat are apparently the principal factors contributing to the difference between countries of origin of the bacons. Comparison of the mean values of Table XIII with the significant differences demonstrated by the variance analysis shows that the Danish product is superior in respect of the corresponding attributes of quality. In general, the results from the Lyons' jury group are in agreement with those from the experiments discussed previously but have the advantage that the reasons for the Danish superiority can be somewhat more clearly defined.

Acknowledgments

These investigations were made possible by the financial assistance received from the Department of Agriculture through Mr. A. M. Shaw, Director of Marketing Service. The Department of Trade and Commerce also provided funds for part of the assistance given by the McLaren Advertising Agency under the direction of Mr. W. G. Abel. The successful completion of a large part of the work must be attributed to the assistance of the McLaren Advertising Agency and Mr. Abel. Not only did this firm provide the necessary office facilities and staff for preparing and mailing several thousand score cards, letters of instructions, etc., but they also enlisted people as tasters, assisted in preparing and distributing the samples, and collected and summarized the results. Apart from the technical assistance, gratitude is accorded Mr. Abel personally for his advice and encouragement. The assistance of other members of Mr. Abel's staff, notably Mr. J. Gellard, Mr. A. Brown, and their associates, is gratefully acknowledged.

To the Department of Scientific and Industrial Research most grateful appreciation is expressed for making available the Smithfield Laboratory in which much of the work was done. Sincere appreciation is expressed to Dr. J. Pace, Officer-in-Charge of this laboratory, for his technical assistance and advice and for his splendid co-operation in providing the necessary facilities.

To the various importing firms, and to Messrs. Impey and Dyke, and Liptons Limited, a debt of gratitude is due for making available experimental materials and members for the jury groups; the firms included are: Ridpath Bros., Loudon and Co., Lane and Co., Sheed-Thomson and Co., and Armour and Co. The services of Mr. Alex Sheed in supervising one of the jury groups are particularly appreciated. To the various people who functioned on

the jury groups, the greatest credit is due for the unselfish way in which they contributed from their time, and for the genuine interest they took in providing conscientious opinions throughout the tests.

The assistance of Dr. R. A. Fisher of University College, London, who outlined the statistical design of most of the experiments, is most gratefully acknowledged. Thanks are also tendered to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for advice and assistance with the final statistical computations.

Dr. W. F. Allen, Agricultural Commissioner, and Dr. G. Miller gave generous assistance and very helpful advice in initiating the work. Mr. Bryan, Trade Commissioner, Liverpool, was most diligent in arranging for tasters, preparing instruction sheets, and delivering samples in the northern provinces. To these individuals sincere appreciation is expressed.

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THE STUDY OF THE EFFECT OF TEMPERATURE UPON PUPATION¹

BY GEOFFREY BEALL²

Abstract

The problem investigated is that of dealing with the cumulative effect of temperature on the progress of the pupation of an insect. There is suggested a statistical model that appears to correspond with physical reality. On the basis of this model, there is suggested an analysis whereby one may determine the effect of temperature upon pupation when insects have been exposed to known varying temperatures and the total period required for pupation is known. The analysis, when applied to data obtained under regularly oscillated temperatures, furnishes a relationship fitting the data.

1. Introductory

Although it is well known that the time spent by an insect in pupation is dependent on the temperature to which the pupa is exposed, the establishment of empirical relationships has been hampered by the peculiar situation that only the aggregate effect of many temperatures, which is known by the duration of pupation, can be observed and, generally, not the effect of a given temperature for a given period. In much experimental work the complexities of the situation have been avoided, to some extent, by dealing with constant temperatures and considering the results per unit period; however, it appears that even this simple situation demands some consideration. We propose to suggest such a quantitative model of the development as will be amenable to statistical test and furnish a basis for the analysis of data obtained under varying temperature.

2. The Measurement of Development

The study of development with respect to temperature has been deeply influenced by the work of Shelford (6) who dealt with the velocity at which a process such as pupation goes forward, velocity being defined as the reciprocal of the time taken to complete the process under constant temperature. Rather than velocity we prefer the concept of the fraction of development or reorganization of a pupa that occurs in a unit period. The two concepts are close and have indeed been used interchangeably, as by Ludwig and Cable (4). In order to visualize reorganization as a sum of additive parts, reorganization, although an intangible quantity, should, as a varying condition, be distinguishable from one time to another and, under constant temperature, be referable to a scale of time; hence stages of the reorganization should correspond to fractions of the total time required for pupation. Specifically, if at the

¹ *Manuscript received for publication December 31, 1940.*

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constant temperature, t_i , the j^{th} pupation occupies n_j unit periods, then in any such period it completes, of its reorganization, a fraction,

$$\varphi_j = 1/n_j \quad (1)$$

Our idea is to refer the progress of pupation to a scale of time just as in educational research the intangible quantity, intelligence, is referred to age.

In carrying out an investigation on development under constant temperature, it is natural to treat a group of pupae, rather than one; assuming that a lot of L_i pupae are exposed to the constant temperature, t_i , and that the j^{th} ($j = 1 \dots L_i$) completes its pupation in n_{ij} unit periods and in one period a fraction, as in Equation (1),

$$\varphi_{ij} = 1/n_{ij} \quad (2)$$

The end of such experimental work as that under discussion is the estimation of the expectation, φ_i , of φ_{ij} , for all possible values of j , customarily made as

$$f'_i = 1/n_i \quad (3)$$

where

$$n_i = 1/L_i \sum_j n_{ij} \quad (4)$$

is the mean period required for pupation in the sample. It is obvious, however, that the estimate of Equation (3) may be replaced by a more direct estimate of φ_i , viz.,

$$f_i = (1/L_i) \sum_j 1/n_{ij} \quad (5)$$

Since, unfortunately, we lack data on individual pupae we cannot determine any differences, $f'_i - f_i$. There is reason, however, to suppose that such differences will vary in magnitude inversely with the magnitude of φ_i , so that if calculation follow Equation (3), the form of the relationship to varying t_i will be affected.

If the concept of a fraction of reorganization, or indeed that of velocity of development, is to be fruitful there must be a certain simplicity in the course of development with time as follows: in pn_{ij} ($0 \leq p \leq 1$) unit periods the j^{th} pupa will reach a definite, perhaps recognizable, point in its reorganization and it must be supposed that always the j^{th} pupa kept at the constant temperature, t'_i , and requiring a total period, n'_{ij} , will attain the same state of reorganization in pn'_{ij} unit periods. Thus we must suppose that the departure from expectation, $\varphi_{ij} - \varphi_i$, in the fraction of development completed in a unit period by a given pupa is characteristic of the whole period of its development. Such a concept is equivalent to saying that among the i^{th} group of pupae, subjected to the temperature, t_i ,

$$\varphi_{ij} = k_{ij}\varphi_i \quad (6)$$

where k_{ij} is a constant peculiar to the j^{th} pupa. From Equation (6) it follows that $\sigma_{\varphi_{ij}}$, the standard deviation of the rate of development over all possible values of j and under a given temperature, t_i , is φ_i of $\sigma_{k_{ij}}$, the standard deviation of a constant peculiar to the j^{th} pupa and independent of the given temperature; thus it has been effectively supposed that $\sigma_{\varphi_{ij}}$ should be pro-

portional to φ_i . We cannot, however, test this point, and hence our primary assumption, directly by an examination of estimates of φ_{ij} because we lack data on individual pupae. From information available, however, on the period of pupation we can determine whether Equation (6) is substantially true. It is well known that in a case such as the present where n_{ij} is by definition the

TABLE I

THE TIME* REQUIRED FOR PUPATION BY MALE *Drosophila melanogaster*, WHEN SUBJECTED TO CONSTANT TEMPERATURE, AN ESTIMATE OF $\sigma_{n_{ij}}$ AND THE APPROXIMATE VALUE OF φ_i .

Temperature, °C.	Number of flies emerging	Pupation in days	$s_{n_{ij}}$	f'_i
15.0	40	14.15 ± 0.036	0.34	0.07
20.0	92	6.78 ± .008	.11	.15
22.5	63	5.27 ± .012	.14	.19
25.0	118	4.26 ± .007	.11	.23
26.0	35	4.00 ± .006	.05	.25
27.0	108	3.78 ± .003	.05	.26
27.5	110	3.65 ± .002	.03	.27
28.0	71	3.56 ± .005	.06	.28
28.5	47	3.45 ± .004	.04	.29
29.0	104	3.40 ± .004	.06	.29
29.5	105	3.35 ± .004	.06	.30
30.0	66	3.35 ± .003	.04	.30
31.0	21	3.35 ± .008	.05	.30
32.0	24	3.50 ± .008	.06	.29
33.0	17	3.63 ± .016	.10	.28

* After Ludwig and Cable (4).

reciprocal of φ_{ij} , with an expectation of φ_i , the standard deviation, $\sigma_{n_{ij}}$, of the period, n_{ij} , of pupation, will be, to a first approximation $1/\varphi_i^2$ that of φ_{ij} . Consequently, if $\sigma_{\varphi_{ij}}$ is proportional to φ_i , then $\sigma_{n_{ij}}$ must vary approximately inversely as φ_i . Estimates of $\sigma_{n_{ij}}$ (actually the estimated probable error of n_{ij}) are given by Ludwig and Cable (4) who reported the performance under constant temperatures of *Drosophila melanogaster* Meigen; their results for males are included in Table I, where, from Equation (3) we have presented f'_i as an estimate of φ_i and $s_{n_{ij}}$ as an estimate of $\sigma_{n_{ij}}$. It can be seen that there is a suggestion that the two estimates vary inversely so that our theory may be true.

3. The Simple Relationship between Development and Variable Temperatures

Generally, the end of work with a number of groups of pupae kept under various constant temperatures, is to obtain estimates of the quantities, φ_i , that may be plotted against the temperature, t_i . For any one group, however, the problem is that of estimating central tendency. Now, however, we wish to deal in a similar way with the development as it occurs under varying temperatures, such as occur naturally; then the problem becomes one of

determining directly a function of temperature. For the moment we shall consider a simple relationship between variable temperature and development in the sense that we shall attempt to explain the effect of variable temperatures as simply an aggregate of the effects of a number of constant temperatures. To a large extent, such a simple relationship is adequate although variability also clearly has other effects, as is discussed in Section 5.

Suppose as in Section 2, that the j^{th} pupation is completed in n_j periods, and that the j^{th} pupa, at the temperature, t_j , of the s^{th} period effects, by analogy with Equation (1), a fraction, φ_{js} , of its total development. When the temperature is not constant, it can be said that

$$\sum_j \varphi_{js} = 1 \quad (7)$$

where the summation is made over the n_j periods occupied by the j^{th} pupation. Equation (7), in which it is known only that the development that occurs in all the periods under the conditions peculiar to each finally results in the completion of pupation, illustrates our initial point, in Section 1, that the effect of temperature can only be seen in the aggregate.

It is necessary to make an estimate of a general fraction, φ_s , of development in the s^{th} period, such that, on the substitution of φ_s for φ_{js} , Equation (7) will be satisfied as nearly as possible for all values of j . Let us assume that there may be obtained an estimate, F_s , of φ_s , which when substituted for φ_{js} in Equation (7) will yield a discrepancy,

$$\xi_j = 1 - \sum_j F_{js} \quad (8)$$

and that F_{js} may be chosen so that $\sum_j \xi_j^2$ is minimal. Now as indicated above, φ_s must be a function of t_s . Guided by general experience with the quantities, φ_i , it may be assumed that φ_s does not vary abruptly with changes in temperature and, further, that

$$\varphi_s = a_0 + a_1 t_s + a_2 t_s^2 + a_3 t_s^3 + \dots \quad (9)$$

where a_0 , a_1 etc. are constants. From Equation (9) the minimization of $\sum_j \xi_j^2$ will be straightforward. It will be necessary ultimately to gain some experience with respect to the nature of the variability of the quantities with which we are dealing when perhaps some other form of estimation will be found more suitable.

4. The Effect of Regularly Oscillating Temperatures

In investigations on the effect of temperature upon the course of pupation there has been a natural tendency to study the effect of oscillating temperatures as compared with the effect of a temperature that is the mid-point of the oscillations. The problem and its possible solution are illustrated below with the data of Mikulski (5). He kept 14 groups of pupae of *Tribolium confusum* Duval at various temperatures and recorded the mean time spent in pupation by each group as is shown in our Table III. Let us deal with time in half-days, since Mikulski varied the temperature at such intervals, and assume that the i^{th} group of pupae completed pupation in n_i half-days

for unfortunately, these records must be treated as if the mean result for a given group of pupae obtained for a single, i^{th} , pupa; this is not strictly correct when applying the foregoing discussion.

We shall not proceed to elaborate the general solution for variable temperatures as indicated in Section 3 because of a modification necessitated by Mikulski's presentation. First, note that he restricted his treatments to subjection of the pupae either to a constant temperature, or to two alternating temperatures, e.g., for the i^{th} group, these temperatures were $T \pm R$, and that each group of pupae was distinguished by a peculiar pair of values of T and R . Write as before, the fraction of development completed ideally in a half-day at the temperature, $T \pm R$, as $\varphi_{T \pm R}$ with, as in Equation (9),

$$\varphi_{T \pm R} = a_0 + a_1(T \pm R) + a_2(T \pm R)^2 + a_3(T \pm R)^3 + \dots \quad (10)$$

Then for an entire day, assume that the fraction of development was

$$\begin{aligned} \varphi_{TR} &= \varphi_{T+R} + \varphi_{T-R} \\ &= 2a_0 + 2a_1T + 2a_2(T^2 + R^2) + 2a_3(T^3 + 3TR^2) + \dots \end{aligned} \quad (11)$$

From Mikulski's discussion of his experimental procedure it is not clear with which temperature, of a given pair, he commenced so that we cannot distinguish between the two temperatures and must suppose that the pupae were exposed equally to each temperature, as must have been approximately the case. Since development was completed in $n_i/2$ days, we can write, as in Equation (7),

$$n_i \varphi_{TR}/2 = 1 \quad (12)$$

Then, as was discussed in connection with Equation (8) we can make an estimate, F_{TR} , of φ_{TR} by getting from Equation (11) and Equation (12), by the method of least squares, for all observed values of i :

$$\left. \begin{aligned} a_0 \sum_i n_i^2 + a_1 \sum_i n_i^2 T + a_2 \sum_i n_i^2 (T^2 + R^2) + \dots &= \sum_i n_i \\ a_0 \sum_i n_i^2 T + a_1 \sum_i n_i^2 T^2 + \dots &= \sum_i n_i T \\ \dots &\dots \end{aligned} \right] \quad (13)$$

and solving for a_0, a_1 etc.

Let us consider the number of constants that will be necessary in estimating φ_{TR} for the present data. Since inspection of Mikulski's results suggests that $\varphi_{TR}|_{R=0}$ will probably tend to approach the T -axis gradually, as T diminishes, and tend to maximize for the greatest values of T used in the experimental work, we may suppose that a third order equation, with four constants, will represent Equation (10) and hence Equation (11) in a satisfactory manner. As a test, we compared the sum of the squared deviations about the relationship, F_{TR} , when fitted with four constants (10 degrees of freedom), i.e., 0.0668, with the corresponding figure, 0.0635 obtained from five constants; from this work it was apparent that the third order was adequate. The estimates of the constants, a_0, a_1 etc., for Equation (11), of course, apply equally to Equation (10) and we choose to present them in the

latter connection, writing that generally for any temperature, $T \pm R$, acting for a half-day, the fraction of development was

$$F_{T \pm R} = +0.313,886 - 0.042,944,3 (T \pm R) + 0.001,896,969 (T \pm R)^2 - 0.000,024,134,5 (T \pm R)^3 \quad (14)$$

For the range of temperatures, in degrees Centigrade, covered by Mikulski, the relationship, Equation (14), is shown in Table II and in Fig. 1, where it can be seen that the relationship is sigmoid as was anticipated.

TABLE II

THE FUNCTION OF EQUATION (14) TABLED FOR THE RANGE OF THE EXPERIMENTAL WORK

$T \pm R$	18	19	20	21	22	23	24	25	26
$F_{T \pm R}$.015	.017	.021	.025	.030	.036	.042	.049	.055
$T \pm R$	27	28	29	30	31	32	33	34	35
$F_{T \pm R}$.062	.069	.075	.081	.087	.091	.095	.098	.100

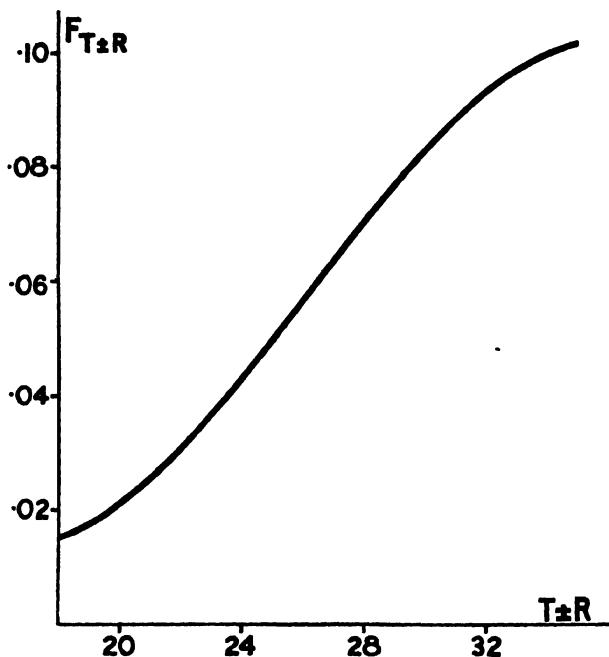


FIG. 1. The fraction, $F_{T \pm R}$, of pupation completed in half a day at a temperature, $T \pm R$, as in Equation (14) and Table II.

As a verification of our work, we obtained, by using the constants from Equation (14) in Equation (11), estimates of the fraction, F_{TR} , of the development that should be completed in one day under the conditions of Mikulski's experiment and contrasted this quantity with the fraction observed to have been completed, i.e., $f'_{TR} = 1/n_i$, as shown in Table III, where it can be seen that a fair correspondence exists.

TABLE III

THE CALCULATED AND OBSERVED FRACTIONS OF DEVELOPMENT COMPLETED IN ONE DAY WITH THE VARIOUS COMBINATIONS OF ALTERNATING TEMPERATURE USED BY MIKULSKI

$T - R$	22.5	20.0	17.5	25.0	22.5	20.0	17.5	27.5	25.0	22.5	20.0	30.0	27.5	25.0
$T + R$	22.5	25.0	27.5	25.0	27.5	30.0	32.5	27.5	30.0	32.5	35.0	30.0	32.5	35.0
n_i	14.40	13.67	13.32	9.90	11.88	9.34	8.90	6.70	8.08	8.30	8.30	5.90	6.06	6.90
f'_{TR}	.069	.073	.075	.101	.084	.107	.112	.149	.124	.120	.120	.169	.165	.145
F_{TR}	.066	.069	.080	.098	.099	.102	.107	.131	.130	.126	.121	.162	.159	.149

5. The Effect of Variability in Temperature

Mikulski was interested particularly in the effect of variability in temperature on development. In our terms, Mikulski's discussion on the difference between the rate of development under oscillating temperatures and the rate under a constant temperature (the mid-point of the oscillations) can be expressed as an investigation for a given value of T , on

$$d_{TR} = f'_{TR}|_{R \neq 0} - f'_{TR}|_{R=0} \quad (15)$$

To d_{TR} we may contrast, for a given value of T , the quantity,

$$\begin{aligned} D_{TR} &= F_{TR}|_{R \neq 0} - F_{TR}|_{R=0} \\ &= (+0.003,793,94 - 0.000,144,807 T)R^2 \end{aligned} \quad (16)$$

by substitution of the values of a_0 , a_1 etc., as in Equation (14) in Equation (11). In Equation (16), of course, $D_{TR} = 0$ when $R = 0$ and when $R \neq 0$, $D_{TR} < 0$ for $T > 26.2$ and $D_{TR} > 0$ for $T < 26.2$. These inequalities are essentially the conclusions on which Mikulski remarked when he showed that pupae subjected to two temperatures, $T \pm R$, did not complete their development in the same time as those subjected to the constant temperature, T . The present work suggests that the effects that he detected could be largely explained by saying that the mean of a curvilinear function at any two values of the independent variable must generally differ from the value of the function at the mid-point of the independent variable. It is probable that to a large extent the problem of the effect of variable temperatures that vexes the ecological literature could be resolved in terms of a simple curvilinear relationship, as in Fig. 1, between the fraction of development, completed in a unit period, and temperature.

Inasmuch as evidence of a directly experimental nature has generally shown that the response of an organism to a given temperature is conditioned by its previous experience of temperatures, there undoubtedly exist some effects that cannot be explained in terms of our simple relationship. A particularly clear case of a true effect of varying temperatures is furnished in a related field by Buchanan (3) who showed that the development of embryos of *Amblystoma punctatum* under oscillating temperatures depended clearly on the period of the oscillations. Since there is no room in terms of our simple relationship of Section 3, for the period of oscillation to have any effect, variability clearly has a proper effect. In the investigation, however, of

such true effects of variability, allowance should clearly be made first for the curvilinearity of the simple effect.

In order to establish an empirical relationship for an effect truly ascribable, in the sense just discussed, to variability in the temperature, one might perhaps, instead of saying, as in Equation (9), that development is $f(t_s)$, consider it as $f(t_s, t_{s-1})$, where t_{s-1} is the temperature of the period preceding the s^{th} . A relationship of the type suggested was fitted by Beall (1) for the activity of moths of *Pyrausta nubilalis* Hubn. and it was found that the influence of the preceding temperature was important in modifying the response to the temperature of a given period.

6. Summary and Discussion

We have attempted to build a model of the development of a pupa as a sum of additive parts gauged by time and there is some evidence in the literature that this model is adequate, although more data are required on the statistical nature of development. Incidentally, it appears that in experiments with constant temperatures, the development per unit period should be estimated by using an harmonic mean rather than the arithmetic mean usually employed. On the basis of our model, it should be possible to determine development as a function of temperature when one knows only the total time taken by pupation under variable temperature. We have suggested that such a function may be fitted by the method of least squares and illustrated the fitting on the special case of oscillating temperatures. In this case, the difference in the effect of oscillating temperatures, from that of a constant temperature (the mid-point of the oscillations) can be ascribed to curvilinearity of the response to temperature.

The present theory should not be restricted to the problems of pupation, since in connection with the duration of a larval stage or the development of an egg there must arise the same problems. Further, similar problems may arise in, for example, studying the relationship between the growth of fish and temperature when it is only convenient to measure the fish at long intervals, whereas temperature can be easily determined at intervals as short as desired. The methods suggested in the present paper may be an aid in analysing general or fundamental studies and of direct use in establishing an empirical relationship between development and environmental conditions when animals are being reared in great numbers so that a nice adjustment of conditions would be profitable.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

JULY, 1941

NUMBER 7

STORAGE STUDIES ON LIQUID BLOOD¹

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Abstract

The effect of the addition of glucose, storage temperature, exclusion of air, and constant rotation on the haemolysis of human blood was investigated. Added glucose had the most marked inhibitory effect of any of the factors studied. Addition of glucose results in a pH drift to the acid side; the extent of this drift is governed by storage temperature. Buffering of blood mixtures indicates that the beneficial effect of glucose is only partially dependent upon the pH changes produced. Storage temperature affected also the rate of haemolysis. Within the temperature range -4° to 12.5° C., minimum haemolysis occurred between 2.5° and 6° C. Excluding air and keeping the blood in slow motion also had highly significant effects in the reduction of haemolysis during the first four weeks in storage.

Other factors studied less extensively included differences between donors, dilutions, container sizes, and air pressures. Donor differences are important when the rate of haemolysis is low, but at higher rates they are masked by treatment effects. Dilution with isotonic solutions retards haemolysis markedly. Haemolysis occurred to the same extent in small and large containers. The shape of the container and its position during storage had no demonstrable effect. Preliminary storage tests at pressures of 0 to 350 mm. of mercury above atmospheric pressure indicated that storage at the normal blood pressure may be of some value in reducing haemolysis. Cell volume changes appear to depend on storage temperature.

Introduction

An investigation to determine the best storage temperature for liquid blood was referred to us by the Subcommittee on Blood Storage of the Associate Committee on Medical Research, since unique cold storage facilities were available in the laboratories devoted to food storage and transport investigations. It is generally stated and accepted that blood kept at room temperature haemolyzes more rapidly than blood kept at lower temperatures, but experimental evidence on this point appears to be limited (5). In practice blood is usually stored in the region of 0° to 6° C. (1, 2, 3). The temperature range from -4° to 12.5° C. was studied in this investigation.

Although the primary object was a study of storage temperature, several other factors were investigated. This made it possible to assess not only the effect of storage temperature when other conditions varied, but also the

¹ Manuscript received April 24, 1941.

Contribution from the Division of Biology and Agriculture of the National Research Council, Ottawa. N.R.C. No. 999.

With some financial support from the Subcommittee on Blood Storage of the Associate Committee on Medical Research.

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influence of temperature in relation to the effect of other factors. The choice of conditions other than temperature was determined largely by an effort to approximate certain body conditions. Accordingly the blood was kept in motion, air was excluded, glucose added, and finally pressure applied. Portions of this work were confirmatory in nature; Rous and Turner (10) demonstrated that glucose inhibited haemolysis; DeGowin, Harris, and Plass (4) recently reported "the remarkable inhibition of haemolysis under anaerobic conditions".

Materials and Methods

The effect of the various treatments on the quality of the blood was determined by measuring the degree of haemolysis. Although this criterion is commonly used for estimating the suitability of blood for transfusion, it is recognized that it may not be entirely adequate. Nevertheless, haemolysis is one of the changes that renders stored blood unfit for transfusion, and since it can be measured accurately and quickly, it is well suited to routine studies of this sort.

The number of cells and their fragility were determined in the early experiments but were later abandoned as these two criteria were too insensitive to distinguish between many of the treatments. Measurements of total and oxyhaemoglobin, cell volume, and pH were made on all samples. Sterility tests were also made on the majority of the samples.

Preliminary experiments were made with sheep's blood but the resistance of the sheep cell to haemolysis made it unsuitable for the present studies. Even after 12 weeks' storage the haemolysis was less than 0.5%. Sheep's blood was also difficult to sample and analyse accurately owing to the formation of clots during storage. Human blood was therefore used in subsequent experiments.

Blood was drawn from healthy male adults in amounts of 200 to 300 ml. in flasks containing the appropriate solutions. The resulting blood mixture was then apportioned directly into storage tubes of about 10 ml. capacity. Aseptic precautions were taken at all stages until the blood was removed from storage. In most of the experiments samples were withdrawn from storage at weekly intervals.

On removal from storage the cells were resuspended by careful shaking, and samples were removed for haemoglobin, cell fragility, and sterility tests. The remainder of the sample was centrifuged under standard conditions and the cell volume estimated. The plasma was then removed for analysis, and pH measurements made on the remaining materials, principally red cells, with a glass electrode.

The degree of haemolysis and the amount of total and oxyhaemoglobin were determined with an Evelyn photoelectric colorimeter (6). Analyses of whole blood (plus anticoagulant) were made after dilution to 0.2%. The haemoglobin in the supernatant plasma (haemolysis) was measured after dilution to 50 or 10%, according to the degree of haemolysis. These dilutions

insured that all observations were made in the central range of the colorimeter scale where errors are minimal. With the small samples available this procedure necessitated in certain instances the use of the "micro" attachment for the Evelyn instrument. When the colour was determined, using the 540 filter employed for oxyhaemoglobin, the plasma of freshly drawn blood always showed detectable absorption. This absorption could not be demonstrated with certainty in plasma dilutions comparable to those made with whole blood.

TABLE I

PLASMA COLOUR AFTER ONE DAY'S STORAGE CALCULATED AS MG. HAEMOGLOBIN PER 100 ML.

All samples rolled with glucose in completely filled tubes. Single observations.				
Donors	Storage temperature, °C.			Mean
	0	5	10	
G.P.	41	34	38	38
A.L.	39	41	43	41
E.C.	72	57	66	65
R.M.	72	72	81	75
Mean	56	51	57	55

Table I shows that the plasma colour after one day's storage, calculated as haemoglobin, differs for different donors. Certain information indicated that part of this plasma colour might be due to dissolved haemoglobin, and, lacking definite evidence to the contrary, it was expressed as haemolysis. Initial variations of this sort, as well as variations in the haemoglobin contents of the blood due to donor differences and differential dilution, all affect the degree of haemolysis if it is expressed as a percentage of the total haemoglobin present. In these circumstances it seemed desirable to express the haemolysis directly as the mg. dissolved haemoglobin per 100 ml. of blood. In any event the statistical design of all these experiments prevented the initial colour or concentration of haemoglobin from being confused with treatment effects.

Although it would be desirable to maintain the same sodium citrate concentration, isotonicity, and dilution in all samples, this becomes impossible if glucose is to be added to certain samples and not to others. When glucose is added the tonicity or dilution must be changed. The results of some initial experiments on the effects of container shape and size, reported later, showed that dilution with isotonic solutions caused a marked decrease in haemolysis during storage. Since this tended to increase the storage period required to distinguish between the treatments under investigation, an attempt was made to maintain the same dilution and final sodium citrate concentration, and accept a somewhat hypertonic solution. Some workers have found less haemolysis following the use of hypertonic diluents (9). When glucose was not added, nine volumes of blood were added to one volume of 3.8% (isotonic)

anhydrous sodium citrate. The final samples were therefore isotonic and contained 0.38% citrate. When required, the necessary amount of a 50% glucose solution, sterilized by filtration, was added to this citrated mixture to make the final mixture 2.8% glucose. Since the volume of added glucose was small, the dilution and citrate concentration were not changed appreciably, and the final osmotic pressure corresponded to 1.5 times that of an isotonic solution. Before adding the 50% glucose solution the cells were allowed to settle and the glucose solution added to the supernatant plasma. This procedure caused no detectable haemolysis, whereas the direct addition of concentrated glucose haemolyzed some of the cells.

During storage the blood was held under conditions that maintained the reported temperature well within $\pm 0.1^\circ \text{C}$. in the majority of instances. In some tests, however, the temperature occasionally fluctuated as much as $\pm 0.4^\circ \text{C}$. Some blood samples were kept in motion during storage; this was accomplished by rotating the tubes about their horizontal axis at a rate that kept the cells in suspension (3 rev. per hr.). Stationary tubes were kept in a vertical position, since the container experiments showed no differences due to the position of the tubes. Tubes to be completely filled were drawn down at one end and provided with a short length of rubber tubing. After they were filled and stoppered aseptically, they were inverted, the retained air bubble was trapped in the tubing, and the tubing clamped off next to the tube. The partly filled tubes had an air space of 3 to 4 ml. between the 10 ml. of blood and the stopper. According to DeGowin, Harris, and Plass (5) blood in tubes stoppered in this way behaves similarly to blood stored in tubes plugged with cotton.

Results

Container Experiments

Prior to the main part of the investigation some work was undertaken to obtain information on the effect of the shape of the storage vessel, shaking, air space in the container during storage, and subsequent aeration. The initial experiments were made with citrated bovine blood held in 300 ml. containers. Containers of two shapes were used, Florence flasks and tubes 1.5 in. in diameter and 12 to 14 in. long. In half the containers about 10% of the volume was air space whereas the other half were completely filled. Duplicate sets of these vessels were held in both horizontal and vertical positions during storage and shaking. As soon as the blood had reached the storage temperature (0°C .), the samples to be shaken were oscillated at 116 cycles per min. for five hours and, after two weeks' storage, were given a similar treatment. At the end of the storage period the samples were heated to 37°C . and two litres of air bubbled through the blood for a period of one hour.

The results of haemolysis measurements made at each stage are reported in Table II and a statistical analysis of these in Table III. Completely filling the containers reduced haemolysis significantly. Shaking, particularly after storage, and aeration increased haemolysis, presumably owing to increased

cell fragility. Indications are that the differences due to the shape of the container and its position during storage are insignificant.

Later experiments on the effect of container size were rather unsatisfactory owing to the fact that the bovine blood used clotted when stored for longer than two weeks. For this reason the experiments were repeated (300 ml. compared with 10 ml.), using human blood diluted (4 to 15) with isotonic dextrose and citrate in the following proportions: blood, 4 parts; dextrose, 10 parts; citrate, 1 part. The storage chamber was held at about 3.0° C.

TABLE II

HAEMOLYSIS OF BOVINE BLOOD AS AFFECTED BY CONTAINER AND TREATMENT,
MG. PER 100 ML.

	Shape and position			Average
	Round	Long vertical	Long horizontal	
Free space	45*	48	54	49.0
No free space	42	44	40	42.0
Average	43.5	46.0	47.0	45.5

	Stage				Average
	Storage for 3 days	Storage for 14 days	Storage for 14 days plus 5 hours' additional shaking	Storage for 15 days plus aeration	
Standing	28**	38	—†	60	41.0
Shaken	24	38	58	79	49.8
Average	26.0	38.0	48.0	69.5	45.4

* Figures represent means of eight analyses.

** Figures represent means of six analyses.

† Value carried over from preceding stage.

TABLE III

ANALYSIS OF VARIANCE OF THE HAEMOLYSIS OF BOVINE
BLOOD AS AFFECTED BY CONTAINER AND TREATMENT

Source of variance	Degrees of freedom	Mean square
Shape	2	47
Space	1	488*
Stage	3	4121**
Shaking	1	892**
Shape × space	2	154
Stage × shaking	3	489**
Error (Residual)	32	66

* Surpasses 5% level of significance.

** Surpasses 1% level of significance.

A table of means and the corresponding analysis of variance is presented in Table IV. The efficacy of dilution in reducing haemolysis (8) is shown by the fact that after seven and one-half weeks' storage, the haemolysis of these samples, corrected for dilution differences, is comparable with that developed after two weeks' storage in concentrated samples (Fig. 1). The main point of interest is that no difference due to the size of the container can be demonstrated. The absence of free space was slightly beneficial, but not significantly so. The long storage period reversed the beneficial effect of rolling over shorter periods, reported later, presumably because of increased cell fragility. The main difference emerging from this experiment was that between donors.

TABLE IV
HAEMOLYSIS AS AFFECTED BY TREATMENT

Treatment means	
Source of variance	Haemolysis, mg. per 100 ml. Means of eight observations
Large container, 300 ml.	42
Small container, 10 ml.	43
Completely filled tubes	41
Free space	44
Rolling	47
Standing	38
Donor F.S.	49
Donor T.S.	36

Analysis of variance		
Source of variance	Degrees of freedom	Mean square
Container size	1	3.1
Free space	1	33.1
Rolling	1	351.6**
Donors	1	637.6**
Error (Residual)	11	23.7

** Surpasses 1% level of significance.

Cell Counts

Typical red and white cell counts obtained after various treatments and storage conditions appear in Table V. There was considerable variation in the number of red cells in the same blood from time to time, probably owing to difficulties of mixing and sampling. Indeed, these variations were larger

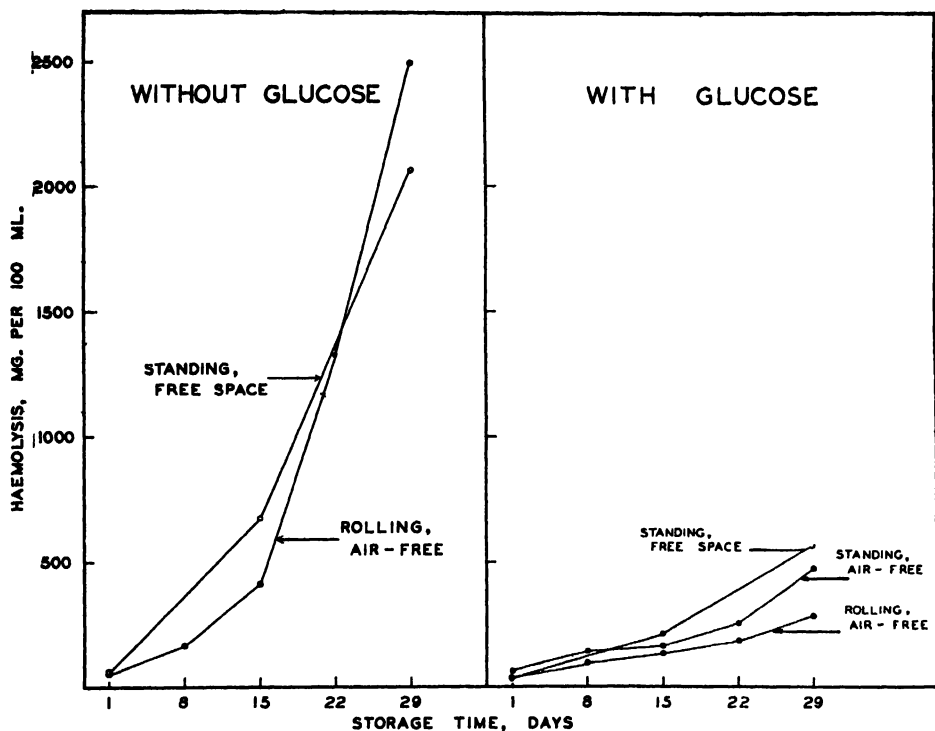


FIG. 1. Successive reductions in haemolysis obtained by adding glucose, excluding air, and keeping the blood in motion.

than were required to account for the maximum haemolysis observed colorimetrically. Since there was no definite trend, it is concluded that none of the treatments or storage conditions caused any significant change in the number of red cells.

The results in Table V show that the number of white cells decreased under all storage conditions tested. This decrease was greatest at the low storage temperatures, and least at 10°C ., the highest temperature employed for this test. Glucose appears to accelerate the destruction of white cells.

Cell Fragility

There was a definite increase in cell fragility under all conditions of storage (Table V). Cells stored at 5°C . were the least, and those held at 10°C . the most fragile. Storage at 0°C . resulted in an intermediate fragility. Other storage temperatures were not included in these tests. When glucose was added the cells were more fragile during the first two weeks of storage, but after that the fragility of blood cells stored with and without glucose appeared to be about the same. During this period no differences in cell fragility could be detected between samples kept in motion and stationary samples.

TABLE V
CHANGES IN CELL COUNT AND CELL FRAGILITY DURING STORAGE

Donor	Temp., °C.	Time, days	R.B.C. (millions)	W.B.C. (thousands)	Fragility		
Glucose, stationary, completely filled containers							
G.P.	10	1	5.40	4.25	0.60 - 0.55*		
		3	5.12	4.10	.60 - .55		
		8	4.68	3.75	.65 - .60		
		15	4.73	3.75	.70 - .65		
		22	5.52	2.25	.85 - .75		
	5	1	4.48	5.05	.65 - .60		
		3	4.96	4.75	.65 - .60		
		8	4.19	2.40	.65 - .60		
		15	4.55	1.20	.65 - .60		
		22	4.86	1.05	.80 - .70		
	0	1	4.84	4.50	.65 - .60		
		3	5.30	3.00	.65 - .60		
		8	4.10	1.75	.70 - .60		
		15	4.32	.15	.70 - .60		
		22	4.65	.05	.80 - .70		
	No glucose, rolled, completely filled containers						
	A.L.	10	1	6.40	5.50	.45 - .40	
			2	6.00	6.00	.45 - .40	
4			5.20	5.00	.50 - .45		
8			5.20	4.90	.55 - .50		
29			5.18	4.00	— - —		
5		1	4.24	5.10	.45 - .40		
		2	5.13	6.20	.45 - .40		
		8	4.30	2.25	.50 - .45		
		Glucose, rolled, completely filled containers					
		A.L.	10	1	6.87	7.50	.60 - .50
2	5.82			6.40	.60 - .55		
8	5.37			3.80	.65 - .60		
29	5.28			2.15			
5	1		6.62	6.25	.60 - .50		
	2		5.48	6.00	.60 - .55		
	8		4.84	1.60	.60 - .55		

* First figure is the highest concentration of saline in which partial haemolysis occurred.
Second figure is highest concentration in which haemolysis was complete.

R.B.C. = Red blood cells.

W.B.C. = White blood cells.

TABLE VI
OXYHAEMOGLOBIN, GM. PER 100 ML.

All samples rolled with glucose in completely filled tubes. Means of four observations.

Storage time, days	Temperature, °C.			Average
	0	5	10	
1	13.6	13.3	13.4	13.5
8	13.5	13.5	13.3	13.4
15	13.4	13.0	12.8	13.1
22	13.2	12.9	12.7	12.9
29	13.2	12.8	12.6	12.8
Average	13.4	13.1	13.0	13.15

Total and Oxyhaemoglobin

There was some time-to-time variability in the total and oxyhaemoglobin contents of the blood from the same donor. These were generally small and attributable in part to difficulties of mixing and analysing the stored blood. Nevertheless, the means show some tendency for the haemoglobin to decrease during storage, particularly at the higher storage temperatures, and these decrements (Table VI) usually attained statistical significance. Since the contents of total and oxyhaemoglobin were closely correlated in most samples, only the results for the oxyhaemoglobin are reported in Table VI. These measurements were made simultaneously with those for haemolysis, and were taken from samples kept in motion during storage since the errors of sampling were least for such material.

Haemolysis

The first experiment on storage temperature contrasted the effects of 0° and 5° C. over periods up to 31 days. Glucose was added and both air free and partly filled tubes were represented. Samples stored in the stationary condition were compared with others kept in motion by rotation.

The average values for haemolysis, as mg. per 100 ml., for each condition appear in Table VII. The main points of interest were: that less haemolysis occurred at 5° C., and that both exclusion of air and rotation during storage reduced the degree of haemolysis. The statistical analysis given in the second part of Table VII shows that these differences were statistically significant.

These results led to a more extensive study, subsequently referred to as the main experiment, involving the blood from six donors. This experiment was designed to permit comparison of the following conditions: presence or absence of glucose, moving and stationary storage, and partly and completely filled tubes, in addition to the effects of storage time, storage temperature, and donor differences. The results of this study appear in Figs. 1, 2, 3, and Tables VIII, IX, and X.

TABLE VII
HAEMOLYSIS AS AFFECTED BY TREATMENT. DONOR A.M.

Treatment means	
Source of variance	Haemolysis, mg. per 100 ml.
	Means of 12 observations
Completely filled tubes	240
Free space	287
Rolling	238
Standing	289
5° C.	229
0° C.	298
Storage time, 10 days 24 days 31 days	Means of 8 observations
	167
	287
	337

Analysis of variance		
Source of variance	Degrees of freedom	Mean square
Temperature	1	29,191**
Free space	1	13,301**
Rolling	1	16,172**
Storage time	2	60,654**
Error (Residual)	17	1147

** Surpasses 1% level of significance.

TABLE VIII
HAEMOLYSIS, MG. PER 100 ML.

Means of three observations. All samples standing				
Temp., °C.	Donor M.K. Free space		Donor A.W. Glucose	
	Glucose	No glucose	Completely filled tubes	Free space
0	288	671	292	335
2.5	233	692	176	272
5.0	248	907	195	245
7.5	263	1195	201	248
10.0	330	1193	234	251

Means of five observations. All samples in completely filled tubes						
Temp., °C.	Donor A.L. Rolled		Donor E.C. Glucose		Donor G.P. Glucose	
	Glucose	No glucose	Rolled	Standing	Rolled	Standing
0	172	826	208	213	158	184
5.0	121	680	145	204	116	148
10.0	197	1167	199	239	174	217

TABLE IX

HAEMOLYSIS AS AFFECTED BY DONOR DIFFERENCES

All samples with glucose. Means of 15 observations				
Conditions	Date	Donors	Haemolysis, mg. per 100 ml.	Average of pairs
Standing with free space	22/5/40	A.W.	270	271
		M.K.	272	
Rolled in completely filled tubes	13/5/40	A.L.	163	174
		E.C.	184	
	31/5/40	G.P.	150	153
		R.M.	156	

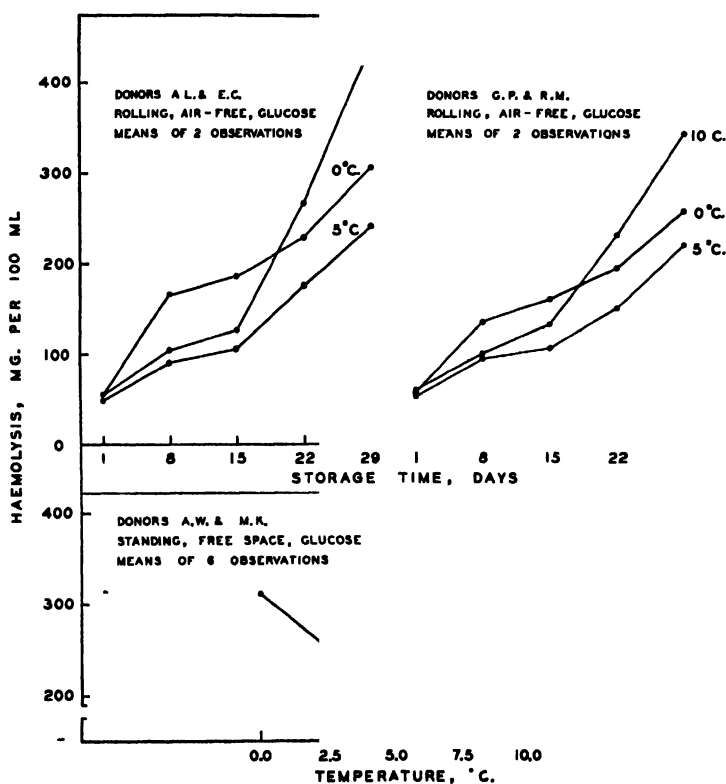


FIG. 2. Effects of storage temperature upon haemolysis, showing differential response with storage time.

In general there was a gradual increase in haemolysis with time in storage. The rate of haemolysis, however, is materially reduced (Fig. 1) by simulating certain body conditions during storage, e.g., addition of glucose, completely filling the containers, and keeping the blood in slow motion. In the absence of glucose (Fig. 1), haemolysis is also reduced by completely filling the con-

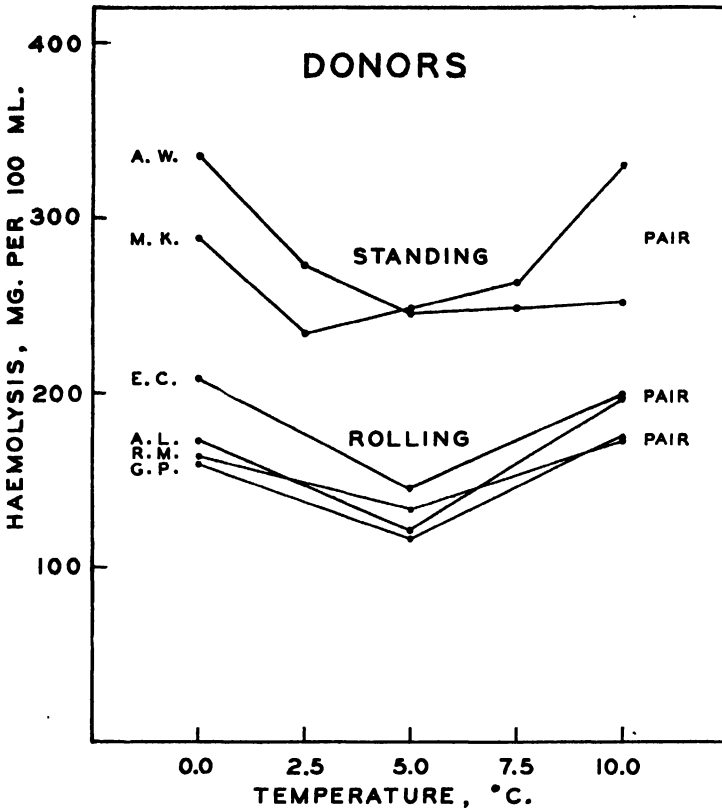


FIG. 3. Donor differences with respect to haemolysis. The standing pair show differential response with storage temperatures.

tainers, and keeping the blood in motion, during the early part of the storage history. On long storage, however, considerable damage is evident, indicating that the mechanical action of rolling may injure fragile cells unprotected by glucose.

The curve in the lower section of Fig. 2 represents an attempt to distinguish between five storage temperatures at 2.5° C. intervals. An analysis of variance failed to distinguish between them. Other tests of significance showed only that 0° C. was inferior to the remaining temperatures. The effect of storage temperature and the differential effects of storage time at different temperatures are shown in the upper section of Fig. 2. Analysis of the results obtained at 0°, 5°, and 10° C., i.e., over 5° C. intervals, showed that 5° C. was significantly better than the 0° and 10° C. storage temperatures in the duplicate tests conducted (Tables VIII and X).

Differences between donors are shown in Tables IX and X(d). The differential effects of temperature on the haemolysis of blood from different donors are shown in Fig. 3. Since the blood samples from this group of donors were treated in pairs, it is possible to obtain a strict comparison

TABLE X(a)

ANALYSIS OF VARIANCE OF HAEMOLYSIS AS AFFECTED BY TREATMENT, WITH SPECIAL REFERENCE TO THE EFFECTS OF KEEPING THE BLOOD IN MOTION

All samples with glucose in completely filled tubes			
Source of variance	Degrees of freedom	Mean square	
		Donor E.C.	Donor G.P.
Rolling	1	9083**	8602**
Storage time	4	102176**	75907**
Temperature	2	5626**	10168**
Storage \times temperature	8	4599**	3342**
Rolling \times storage	4	5495**	2322**
Rolling \times temperature	2	1876**	200
Error (Residual)	8	220	143

** Surpasses 1% level of significance.

TABLE X(b)

ANALYSIS OF VARIANCE OF HAEMOLYSIS AS AFFECTED BY TREATMENT, WITH SPECIAL REFERENCE TO THE INFLUENCE OF GLUCOSE

Source of variance	Samples rolled in completely filled tubes		Samples standing with free space	
	Donor A.L.		Donor M.K.	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Glucose	1	3971968**	1	3261722**
Storage time	4	1932138**	2	4190465**
Temperature	2	204154	4	115158
Storage \times temperature	8	92156	8	72247
Glucose \times storage	4	1263119**	2	1413434**
Glucose \times temperature	2	115556	4	87207
Error (Residual)	8	55762	8	44306

** Surpasses 1% level of significance.

between donors by comparing the samples that were treated identically throughout. Tables IX and X(d) show that the difference between donors A.L. and E.C. is significant, but that the differences within the remaining pairs failed to attain significance. Table IX also shows a difference between pairs treated identically, but collected at different times. In practice this difference is the one most commonly observed. The variation in the initial colour of the plasma from different donors (Table I) bears little relation to the rates of haemolysis obtained after treatment and storage. It will be recalled, however, that in the container study (Table IV), where the rate of haemolysis was rather low, the difference between donors was the greatest difference observed.

TABLE X(c)

ANALYSIS OF VARIANCE OF HAEMOLYSIS AS AFFECTED BY
TREATMENT, WITH SPECIAL REFERENCE TO THE
EFFECTS OF COMPLETELY FILLING THE TUBES

All samples standing with glucose. Donor A.W.		
Source of variance	Degrees of freedom	Mean square
Free space	1	19001**
Storage time	2	536914**
Temperature	4	9396**
Storage \times temperature	8	4119*
Free space \times storage	2	17188**
Free space \times temperature	4	1232
Error (Residual)	8	897

* Surpasses 5% level of significance.

** Surpasses 1% level of significance.

TABLE X(d)

ANALYSIS OF VARIANCE OF HAEMOLYSIS AS AFFECTED BY TREATMENT, WITH SPECIAL REFERENCE
TO DONOR DIFFERENCES

Source of variance	Samples rolled with glucose in completely filled tubes			Samples standing with glucose and free space		
	Degrees of freedom	Mean square			Degrees of freedom	Mean square
		Donors A.L. and E.C.	Donors A.L. and G.P.	Donors G.P. and R.M.	Donors A.W. and M.K.	
Donors	1	3286**	1360	327	1	39
Storage time	4	68536**	63199**	41322**	2	735616**
Temperature	2	12702**	11728**	6308**	4	4876
Storage \times temperature	8	4650**	4210**	1826*	8	4536
Donors \times storage	4	130	517	995	2	2772
Donors \times temperature	2	724	222	213	4	3834
Error (Residual)	8	259	346	364	8	1997

* Surpasses 5% level of significance.

** Surpasses 1% level of significance.

The next experiment was designed primarily to secure further information on storage temperature. Storage temperatures were -4.0° , -2.0° , 0° , 2.5° , 5.0° , 6.0° , 7.5° , 10.0° , and 12.5° C. Glucose was added to give isotonic and slightly hypertonic solutions, since it was felt that a slightly hypertonic mixture might be advantageous. The blood in isotonic and hypertonic glucose was prepared by mixing eight volumes of blood in one volume of isotonic citrate, with one volume of 5.4 or 8.1% glucose, resulting in final

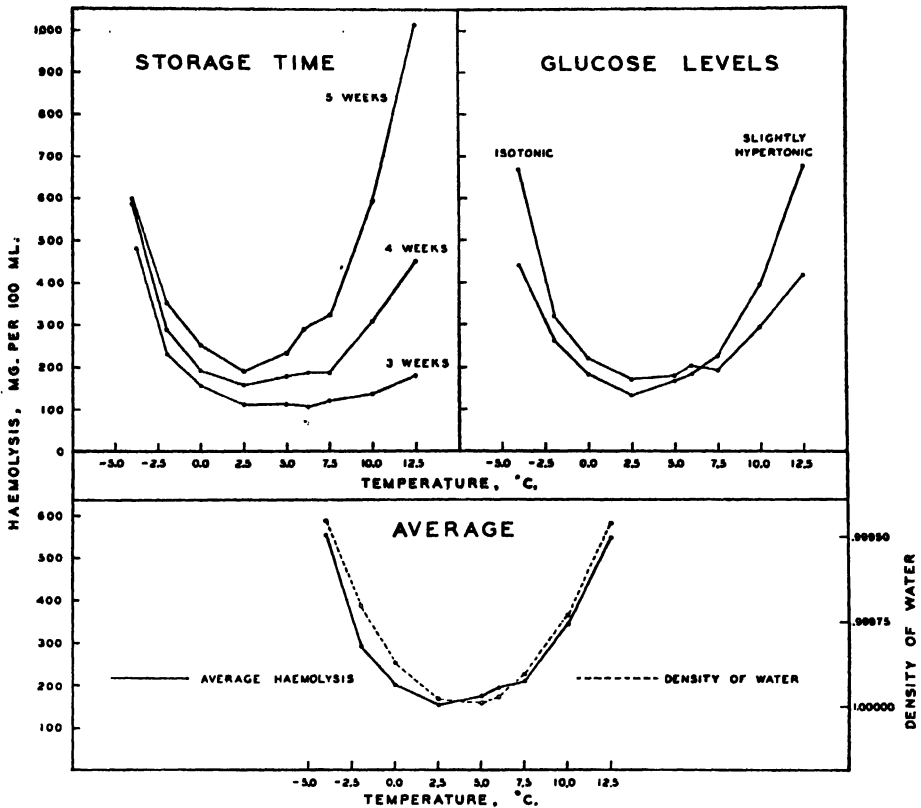


FIG. 4. Haemolysis as affected by storage temperature. The effects of storage time and differing levels of glucose are shown in the component parts of the parabola.

glucose concentrations of 0.54 and 0.81%, respectively. All of the tubes were completely filled and were kept in motion since these conditions had been shown to be effective in reducing haemolysis.

The effect of altering the glucose level of the solution is evident from Fig. 4. Over all temperatures there is no demonstrable effect. However, the slightly hypertonic solution appears to be more effective for reducing haemolysis at the lower range of temperatures studied, whereas the isotonic blood kept better at the higher temperatures.

These results, obtained in approximately isotonic concentration, cannot be compared directly with hypertonic samples in the main experiment (1.5 times the osmotic pressure of an isotonic solution), since the dilution differed in the two series. Average values for a number of comparable storage and other conditions in the two series indicate that the haemolysis for the isotonic and hypertonic mixtures, after proportionality corrections for dilution, was 202 and 255 mg. haemoglobin per 100 ml., respectively. Although this suggests that the isotonic solution is superior, this is believed to be due primarily to the greater dilution used in the isotonic mixture. It has been shown earlier

that the degree of haemolysis decreases with increasing dilution, but this factor was not studied quantitatively.

At temperatures of -4.0°C . and -2.0°C . duplicate samples were stored in both the frozen and supercooled states. The frozen samples were always 80 to 100% haemolyzed; all subsequent figures and discussion of the results obtained at subzero temperatures therefore refer to the supercooled samples.

The influence of storage temperatures is shown by the parabolic curve in Fig. 4, representing the average haemolysis for all storage periods and glucose levels. This curve passes through a long minimum between about 2.5° and 6.0°C .

The influence of storage time is also shown in Fig. 4, by curves obtained for the results after three, four, and five weeks' storage. These curves show that haemolysis accelerates at the higher storage temperatures, confirming the results of experiments previously reported.

The parabolic curve relating haemolysis and storage temperature (Fig. 4) closely approximates the density-temperature curve for water, the principal constituent of blood. The slope of the upper curves in Fig. 4, however, shows that the apparent association is largely fortuitous.

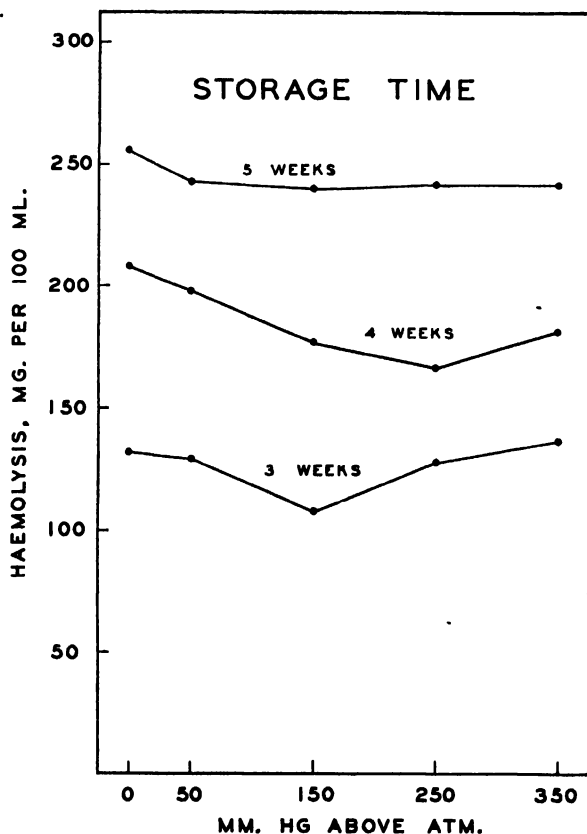


FIG. 5. *The effects of additional pressure upon haemolysis.*

Effects of Pressure on Haemolysis

A preliminary experiment to investigate the effects of pressure upon haemolysis was undertaken near the end of the investigation. Three samples were put under pressure at each of the following levels: atmospheric pressure and 50, 150, 250, and 350 mm. of mercury above atmospheric pressure. For pressure variations due to leakage, which were less than 10 mm., compensation was made by the addition of the necessary amount of mercury in the manometer side-arms. The system was inspected twice daily, and after a few days all leaks were stopped. The room temperature was kept at about 3.0° C.

The results are presented in Fig. 5. One sample at each pressure was removed after three, four, and five weeks' storage. After three weeks the value nearest to blood pressure in the body, 150 mm. of mercury, showed a saving of about 20% of the haemolysis produced under atmospheric con-

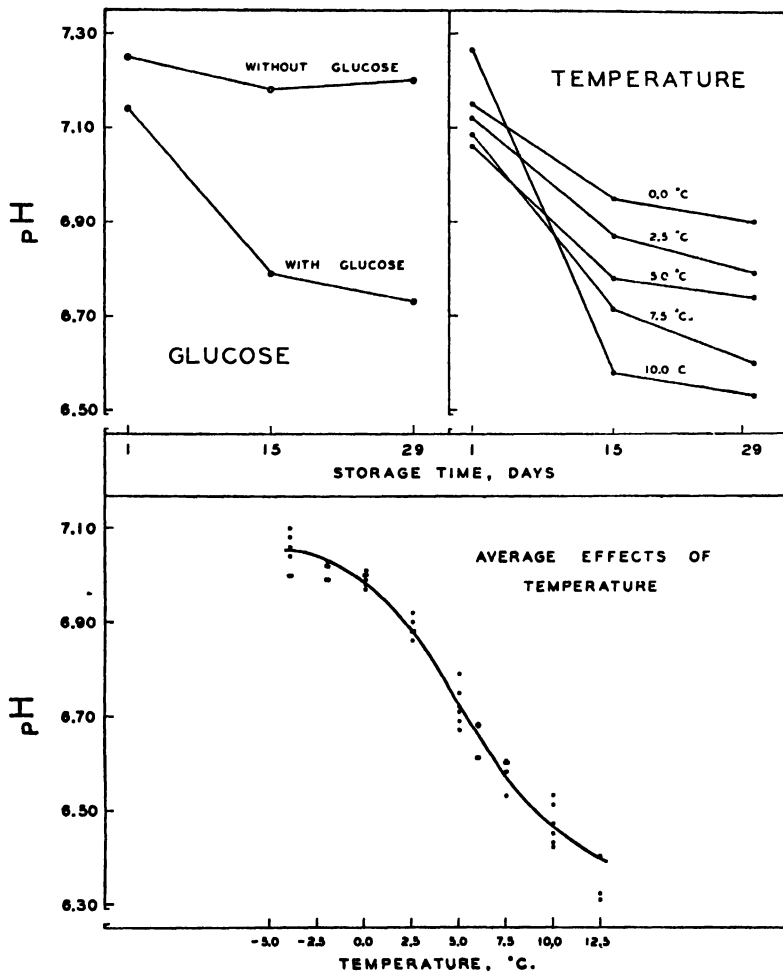


FIG. 6. The pH drift when glucose is added, showing that the extent of drift is dependent upon storage temperature.

ditions. After four weeks, 150 and 250 mm. of mercury are still producing some beneficial effect, but after five weeks the saving of cells under pressure is negligible. The preliminary nature of the experiment and the small number of samples involved made it impossible to demonstrate any statistically significant effect due to pressure. Nevertheless the results are interesting, and suggest that a study on the effects of storing blood under a pressure comparable with that in the body might be of some value.

Changes in pH during Storage

The results of pH measurements made on the samples in the main experiment showed that blood stored without glucose showed no significant pH changes. The addition of glucose, however, causes the pH to drift to the acid side. The extent of this change was greatest at the high temperatures and decreased regularly as the temperature decreased (Fig. 6, upper section). These observations were confirmed by the results of later experiments shown in Fig. 6 (lower section). Minor differences due to storage practice and glucose levels, as well as error, contribute to the scattering of the points. The curve fitted to the points graphically indicates not only that the pH change is greatest at the higher storage temperatures, but also that the slope of this pH-temperature curve is greatest in the temperature range where minimum haemolysis occurs.

These results suggested the use of buffered blood mixtures. Isotonic mixtures of mono- and dibasic sodium phosphate (NaH_2PO_4 and Na_2HPO_4) were used to maintain the pH at higher and lower levels than that of normal blood. In addition to the untreated controls, samples were prepared with

TABLE XI
CHANGES IN pH AND HAEMOLYSIS OF BUFFERED BLOOD SOLUTIONS

pH changes, means of three observations			
Storage time, days	Buffered above normal pH	Unbuffered (normal behaviour)	Buffered below normal pH
0	7.50	7.32	6.50
12	7.30	7.04	6.60
24	7.27	6.96	6.62
36	7.14	6.85	6.59
Haemolysis, mg. per 100 ml., means of three observations			
Glucose level	Buffered above normal pH	Unbuffered (normal behaviour)	Buffered below normal pH
No glucose	756	2551	366
Isotonic glucose	88	88	295
Hypertonic glucose	101	88	268

added glucose in isotonic and slightly hypertonic solutions to represent the condition of changing pH during storage. All samples were stored at 5° C.

The results of the pH measurements appear in Table XI. It can be seen that the pH of the buffered samples changed somewhat during storage, but always remained above or below the pH of unbuffered blood.

The results of haemolysis measurements on these samples are also shown in Table XI. As expected, the presence of glucose markedly inhibits haemolysis. In the absence of glucose, buffering is effective in reducing haemolysis, particularly on the acid side of the normal pH. When glucose is added buffering on the acid side causes increased haemolysis, whereas buffering on the alkaline side of the normal value has little or no effect. The two glucose levels employed appeared to have little influence on the haemolysis.

From these results it appears that the protective action of glucose is not primarily the result of the pH changes it produces during storage. The addition of glucose rather than buffering on the acid side would appear to be the best protection against haemolysis.

Cell Volume

In general the swelling of the erythrocytes on storage paralleled the pH changes, following temperature in the same manner (Fig. 7). The decrease in cell volume at the end of the storage period for the higher temperatures can

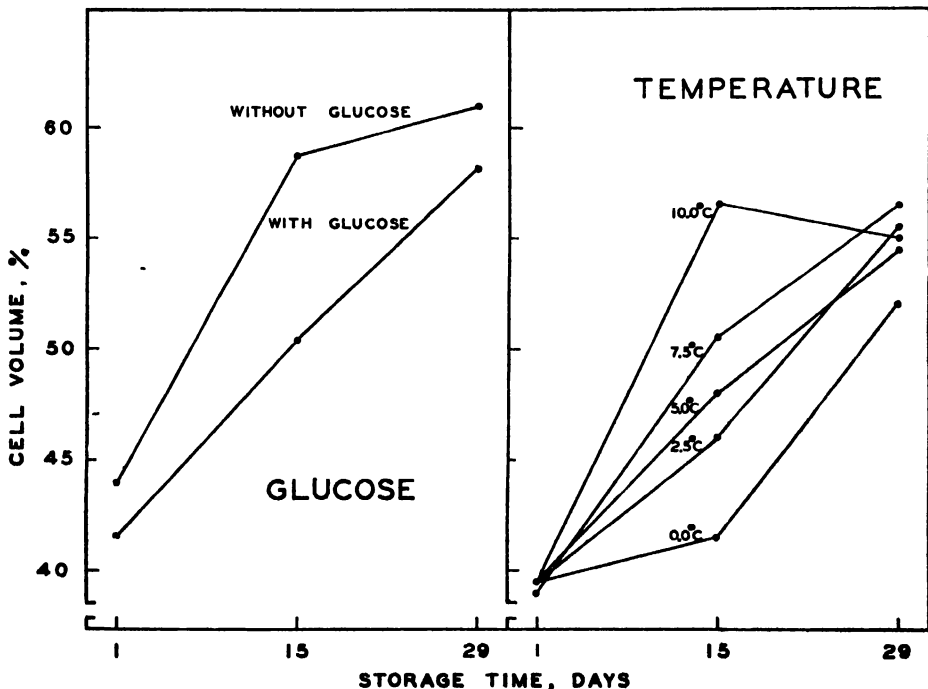


FIG. 7. Cell volume changes. The swelling is governed by storage temperature.

be explained by cell rupture, as is apparent from the greater haemolysis at these temperatures. However, the cells appear to swell faster in the absence of glucose (Fig. 7).

Summary and Conclusions

Minimum haemolysis occurs in the temperature region of 2.5° to 6.0°C. Differences in haemolysis within this range are rather small. A storage temperature of about 4.0° C. might be suggested as the mid-point of the minimum haemolysis range. A differential temperature effect is noted, such that the rate of haemolysis accelerates with time to a greater degree at the higher temperatures studied than at the lower temperatures.

The addition of glucose inhibits haemolysis to a marked extent. Also, the addition of glucose results in a pH drift to the acid side during storage. In general the higher the storage temperature, the greater is the pH drift. Change in pH is most sensitive to temperature in the temperature region associated with minimum haemolysis. Buffering of blood mixtures inhibits haemolysis to some extent when glucose is absent, but in the presence of glucose buffering is either harmful or of no effect, depending on the pH level. This indicates that the beneficial effect of glucose is at best only partially dependent on the pH changes produced.

Keeping the blood in motion in the presence of glucose inhibits haemolysis for the first four weeks of storage. In citrate alone rolling is beneficial for the first two weeks. It would appear to be likely, from the work of Fåhræus (7), that the efficacy of rolling in reducing haemolysis is due to the maintenance of the cell-plasma interface.

Storing the blood in completely filled tubes inhibits haemolysis for the first four weeks of storage.

Some information was obtained on a number of other factors which, however, were not studied extensively. Of those studied, donor differences appeared to be relatively unimportant in comparison with treatment effects when the rate of haemolysis is comparatively rapid. However, when the rate of haemolysis is rather slow, donor differences are an important factor. Dilution with glucose-citrate mixtures in isotonic concentration causes a marked reduction in haemolysis. Over the entire temperature range, no difference in haemolysis could be attributed to slight alterations in glucose concentration. However, there is some evidence that blood, if made slightly hypertonic with glucose, will keep better at the lower temperatures studied. This finding is in line with other investigations (9). A preliminary experiment on the effects of pressure on haemolysis suggests that storing blood under a pressure comparable with that in the body might be of some value.

No difference in haemolysis can be detected when containers of practical dimensions are compared with the sample containers used in this investigation.

Acknowledgments

Grateful acknowledgment is made to the Red Cross Society Transfusion Service, especially to Mr. J. R. Potter, the Secretary, who made arrangements for the medical examinations, and to Dr. S. F. Service and the members of the staff of the Ottawa Civic Hospital who made the examinations. We also wish to acknowledge the assistance of Drs. Rooke and Caldwell, who collected the samples from human donors, and to thank Dr. Paterson, of the Ottawa Civic Hospital, for making their services available.

Mr. A. M. Adams, Laboratory Assistant, was largely responsible for testing sterility, and for making many cell counts and fragility tests. Mr. A. J. McLeod, Laboratory Assistant, also gave valuable technical aid in several phases of the work. Messrs. T. A. Steeves and A. E. Chadderton, of the Biology Division, were of great service in constructing the necessary equipment. Our thanks are also due to the members of the staff of the National Research Council, whose contributions of blood have made the work possible.

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THE ABILITY OF SHEEP'S ERYTHROCYTES TO SURVIVE FREEZING¹

BY A. H. WOODCOCK², M. W. THISTLE³, W. H. COOK⁴, AND
N. E. GIBBONS⁴

Abstract

Erythrocytes from sheep's blood were subjected to several prefreezing treatments, frozen at various rates, and stored at temperatures from $-2^{\circ}\text{C}.$ to $-190^{\circ}\text{C}.$ Minimum haemolysis generally occurred when the cells were suspended in 1 to 1.5 isotonic glucose and frozen extremely rapidly in liquid air. During subsequent storage about two-thirds of the cells survived 32 days' storage at $-190^{\circ}\text{C}.$, only about half survived at $-78^{\circ}\text{C}.$, and haemolysis was practically complete at higher temperatures. Cells dried from the frozen state, *in vacuo*, appeared normal, but always haemolyzed when liquids were added. Cells held in both the supercooled and partly frozen condition at temperatures slightly below the freezing point showed that least haemolysis occurred in the supercooled samples. In the frozen samples the extent of haemolysis increased as the temperature decreased and was practically complete at $-8^{\circ}\text{C}.$ Although the results show that the formation of ice crystals is one of the primary causes of cell disintegration, differences between animals and other unknown factors also have a significant influence on the ability of the cells to withstand freezing.

Introduction

If whole blood or suspensions of red cells in isotonic solutions are frozen, the erythrocytes are practically all destroyed. This has, up to the present time, precluded the use of freezing temperatures for the storage of blood for transfusion or other uses. The object of the present study was to determine the effect of certain pretreatments, rates of freezing, and subsequent holding temperature on the degree of haemolysis of frozen blood.

The results of recent investigations (5, 3) indicate that the formation of ice crystals is a cause of death when living organisms are exposed to freezing temperatures. Whether erythrocytes are considered dead or alive, it may reasonably be assumed that crystal formation is responsible for most of the destructive influence caused by freezing. Consequently, freezing procedures that minimize crystal formation should enable some proportion of red cells to survive.

Extremely rapid freezing rates may pass the material through the temperature range in which rapid crystal formation occurs so quickly that few crystal nuclei are formed. In such a system the water will be present in the vitrified state. Here the practical considerations are whether a suspension of erythrocytes can be frozen at a sufficiently rapid rate, and whether crystal formation in the vitrified mass can be avoided at practical storage temperatures

¹ Manuscript received April 24, 1941.

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over sufficient periods. In this connection it appears (1, 3, 4) that the extent of crystal formation varies directly with the proportion of water to solids in the test material.

Crystal formation can also be reduced by the use of storage temperatures only slightly below the freezing point of the solution. Since blood has a freezing point of about -0.6°C ., whereas the eutectic temperature either is, or could be adjusted to, a much lower value, it follows that the extent of crystal formation depends on the storage temperature. This suggests the possibility of using storage temperatures only slightly below the freezing point. These considerations also indicate that very slow freezing might be beneficial. The crystals produced between the freezing point and the eutectic will be pure water. If these crystals form in the suspending medium, as distinct from the cells, they may not be destructive, and the gradual reduction in the water content of the cells may depress their freezing point sufficiently to avoid freezing or favour vitrification. The experiments were designed to examine all of these possibilities.

Materials

All of these experiments were made with citrated sheep's blood. It was intended that these should be preliminary to similar studies on human blood. Since the results of other investigations (6) have indicated that the erythrocytes from sheep were much more resistant to haemolytic influences than those from humans, it represented better material for differentiating between the various treatments.

Methods

The degree of haemolysis was determined with an Evelyn photoelectric colorimeter (2) and, where necessary, the condition of surviving cells was determined by microscopic examination. Since the oxyhaemoglobin content of the test solutions varied considerably, the haemolysis is subsequently expressed as a percentage of the oxyhaemoglobin present.

Methods for increasing the proportion of solids to water included evaporation and addition of glucose. Preliminary studies showed that blood could be partly dried by evaporation from Visking casings at room temperature. By this procedure a 50% loss of weight was accompanied by no appreciable haemolysis. Glucose in concentrations up to 15% produced little or no haemolysis, but at higher concentrations haemolysis increased rapidly.

Results

Rapid Freezing in Liquid Air

In the first tests a small wire loop was immersed successively in the blood preparation, liquid air, and a thawing solution. Whole blood and blood concentrated by partial drying were thawed in unconcentrated plasma. The results indicated that a 25% moisture loss was the best of these treatments, about 50 to 60% of the cells surviving. Blood cells were also suspended, frozen, and thawed in glucose solutions of various tonicities. The best glucose

concentrations appeared to be between 5.4 and 8.1% (1 and 1.5 isotonic); in these about 75% of the cells survived.

In the next experiments a fine metal screen was immersed in the blood preparation, drained, immersed in liquid air, and stored. The excess liquid air was allowed to boil off at the storage temperature. Samples were stored at -190°C . (liquid air), -78°C . (solid carbon dioxide), -40°C ., and -18°C . The samples were removed after varying periods up to 32 days, thawed by immersion in glucose solutions, and analysed. Blood preparations included whole blood, blood dried to 13 and 30% loss of weight, and cells washed and suspended in 5, 10, 15, 20, 25, and 30% glucose solutions.

The haemolysis resulting from each treatment averaged over all storage treatments and periods is given in Table I. These results show that freezing and thawing in hypertonic glucose solutions is generally better than the other treatments but, on the average, only about 30% of the cells survived.

TABLE I
EFFECT OF FREEZING MEDIUM ON SURVIVAL

Freezing medium	Cells in glucose solutions, %						Whole blood	13% dried	30% dried
	5	10	15	20	25	30			
Average haemolysis, %	72	65	68	68	63	69	74	72.5	72.5

Table II shows the effect of temperature and time of storage averaged over all treatments, and Table III, the results of a statistical analysis of the data for the three lowest temperatures and storage periods up to 18 days. The first analyses were made after one day's storage, and haemolysis indicated at that time includes any that occurred during freezing and thawing as well as storage. Storage over longer periods does cause some additional haemolysis, but storage temperature seems to be the most important factor. At temperatures of -40°C ., or higher, the cells were almost completely haemolyzed,

TABLE II
EFFECT OF STORAGE TIME AND TEMPERATURE ON HAEMOLYSIS OF STORED BLOOD, %

Storage period, days	Storage temperature, $^{\circ}\text{C}$.			
	-190°	-78°	-40°	-18°
1	36.4	41.0	85.0	100
4	37.7	48.0	91.4	100
8	37.2	45.0	95.4	97.5
18	(69.0)	(68.5)	99.0	—
32	35.1	46.0	100.0	—

Note: Values in parentheses doubtful.

at -78°C . about half the cells survived, and at -190°C . nearly two-thirds were intact at the end of 32 days' storage.

TABLE III
ANALYSIS OF VARIANCE OF RESULTS† OF HAEMOLYSIS DURING
FREEZING AND STORAGE

Source of variance	Degrees of freedom	Mean square
Time	3	3043***
Temperature	2	24,666***
Freezing medium	8	420*
Error	93	176

* Denotes 5% level of significance.

*** Denotes 0.1% level of significance.

† Results at -18°C . and those for 32 days' storage not used in this analysis.

From these results it appears that liquid air temperatures would be required to preserve blood in the frozen state. This was considered impracticable, and further investigations designed to reduce the haemolysis observed after one day's storage and attributable in part to the freezing and thawing procedures were abandoned.

Drying from the Frozen State

Since frozen blood cannot be preserved at practicable temperatures, an attempt was made to dry the frozen samples under high vacuum. The blood was prepared as previously described, immersed in a liquid air container, and the entire unit sealed up and the liquid air pumped off in a room at -40°C . Following removal of the liquid air, the water evaporated from the sample under high vacuum and condensed on a series of finned surfaces cooled with liquid air.

Blood cells that had been completely dried in this way appeared normal when examined microscopically. However, all attempts to resuspend these cells in liquid media of various sorts at various rates failed. In these circumstances this line of attack was abandoned.

Partial Freezing

Attention was turned to the storage of partly frozen and supercooled blood at temperatures ranging from -2° to -8°C . Samples (2-ml.) of a mixture of 5 ml. citrated sheep's blood and 20 ml. of 8 or 12% glucose solution were used. All the samples were allowed to attain the desired storage temperatures in controlled baths. One-half of the samples were then seeded with a small ice crystal and the other half allowed to remain in the supercooled state. Below -4°C . the supercooled samples were so unstable that crystallization occurred during storage. Samples were removed for analysis after 1, 8, and 18 days' storage. The results showed that haemolysis increased with time,

the average for the seeded samples being 21.7, 54.0, and 54.0%, and for the supercooled 7.6, 9.3, and 9.5% for 1, 8, and 18 days' storage, respectively. When the results are averaged for all storage periods, it is evident from Table IV that more haemolysis occurred in 12 than in 8% glucose; that haemolysis increased as the storage temperature decreased; and that the degree of haemolysis was usually less in the supercooled than in the frozen samples held at the same temperature. Although these results show that the number of surviving cells decreases as the extent of crystal formation increases, they do not indicate that partial freezing could become a practicable method for preserving suspensions of red cells. Supercooling at temperatures down to $-4^{\circ}\text{C}.$ is better than freezing in the same range, but other studies (6) have shown that storage in the supercooled state causes more haemolysis than storage at about $5^{\circ}\text{C}.$

TABLE IV
HAEMOLYSIS OF SUPERCOOLED AND PARTLY FROZEN CELL
SUSPENSIONS

Temp., $^{\circ}\text{C}.$	State	Haemolysis, %	
		8% glucose	12% glucose
-2	Partially frozen	2.8	7.5
-4	Partially frozen	26.1	38.0
-5	Partially frozen	36.0	56.0
-6.5	Partially frozen	55.0	69.7
-8	Partially frozen	59.3	81.8
-2	Supercooled	1.7	16.2
-4	Supercooled	3.3	16.2

Slow Freezing to $-40^{\circ}\text{C}.$

The results of the experiments described above indicated that the freezing and thawing of blood was accompanied by considerable haemolysis, and even if this could be avoided, considerable difficulty would be experienced in providing the requisite liquid air temperatures for storage. Nevertheless, in order to complete the study, some experiments were undertaken on the effect of freezing rate. In this connection it seemed desirable to cool the samples to a sufficiently low temperature to assure complete freezing and avoid other complications. A final temperature of $-40^{\circ}\text{C}.$ was chosen.

A preliminary experiment showed distinct promise. A 2-ml. sample frozen in air at $-40^{\circ}\text{C}.$ and thawed at room temperature showed only 20% haemolysis. In later experiments the rate of freezing these 2-ml. samples was varied by plunging some tubes in liquid air, then removing them to a room at $-40^{\circ}\text{C}.$, and by placing others in a large volume of ethylene glycol (as thermal ballast) in a Dewar flask and allowing the whole to cool in a room at $-40^{\circ}\text{C}.$ The time required to cool the samples, therefore, was varied from a few seconds to 48 hr. Rates of thawing were varied by exposing some tubes to room temperature (30 min.) and exposing others in ethylene glycol baths originally

at -40°C . to room temperature (48 hr.). These experiments were uniformly unsuccessful, the haemolysis varying from 90 to 100% with no evidence as to the best procedure.

Owing to the marked discrepancy between these results and those of the preliminary experiment, another series of tests was conducted to investigate other variables. These included: a study of blood from four different sheep; effect of storage prior to freezing; and the effect of various glucose concentrations in addition to whole blood. More complete details of procedure appear in Table V, together with the results of the haemolysis measurements. The freezing and thawing procedures were uniform for all samples. Freezing was accomplished by placing 8 ml. in a test tube in a room at -40°C . and thawing by removing to room temperature for one hour. Samples containing hypertonic glucose were brought slowly back to isotonicity by allowing the required amount of water to diffuse into the solution through Visking casings at 5°C .

A statistical analysis of the results of these experiments also appears in Table V. There was a significant difference between the resistances of the erythrocytes of different sheep to these treatments, the average haemolysis

TABLE V

HAEMOLYSIS OF SHEEP'S ERYTHROCYTES FOLLOWING VARIOUS PREFREEZING TREATMENTS AND STORAGE PERIODS AT -40°C .

Prefreezing treatment	Freezing medium	Postfreezing treatment	Haemolysis, %		
			Period of frozen storage		
			1 day	8 days	28 days
1. None	Plasma	None	100	100	100
2. Held in plasma 48 hr.	Plasma	None	99	100	100
3. None	Glucose, 5.4%	None	67.5	77.8	78.5
4. Held in glucose 48 hr.	Glucose, 5.4	None	73.7	87.8	91.5
5. Held in plasma 48 hr.	Glucose, 5.4	None	61.8	70.8	80.0
6. None	Glucose, 5.4	Held 24 hr.	72.8	76.3	77.0
7. Held in glucose 48 hr.	Glucose, 5.4	Held 24 hr.	70.6	85.8	87.3
8. Held in plasma 48 hr.	Glucose, 5.4	Held 24 hr.	60.3	70.7	79.5
9. None	Glucose, 8.1	Returned to isotonicity	51.0	64.8	71.3
10. Held in glucose 48 hr.	Glucose, 8.1	Returned to isotonicity	77.3	84.5	92.5
11. Held in plasma 48 hr.	Glucose, 8.1	Returned to isotonicity	53.5	71.8	80.8

ANALYSIS OF VARIANCE OF RESULTS 6 TO 11 INCLUSIVE, GIVEN ABOVE

Source of variance	Degrees of freedom	Mean square
Concentration of glucose	1	235*
Prefreezing treatment	2	1530***
Storage period	2	1803***
Variance between sheep	3	695***
Error	40	40

* Denotes 5% level of significance.

*** Denotes 0.1% level of significance.

in glucose solutions varying from 68 to 79% for different animals. The effect of the other treatments can best be assessed from the average values obtained from the four sheep. Cells held in glucose for 48 hr. prior to freezing suffered greater haemolysis than cells frozen in glucose within three hours after collecting. On the other hand, cells stored in plasma for 48 hr. prior to freezing in glucose were usually more resistant to haemolysis than the un-stored samples. Cells frozen in plasma, however, were always completely haemolyzed, demonstrating the protective action of glucose at this stage. Haemolysis also increased with increasing time of storage.

Discussion

Since it is outside the scope of this paper to consider the temperature effects of any chemical changes that might produce haemolysis, the matter is discussed from a purely physical point of view. Using the vitrification theory as a working hypothesis it appears that crystal formation produces a breakdown of the erythrocytes. As shown in Table IV minimum haemolysis occurs when the material is supercooled and there are no crystals present. Moreover, haemolysis increases, in the seeded samples, with lowering temperature which is equivalent to greater crystal formation. From the results, shown in Table II, it was suspected that freezing caused some vitrification, and when the temperature was raised recrystallization took place with consequent haemolysis. Also from Tables I and V it appears that hypertonic glucose solutions aid in preserving cells by decreasing the relative water content and inhibiting crystal formation to some degree.

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CANADIAN WILTSHIRE BACON

XX. A COMPARISON OF CERTAIN CHEMICAL AND PHYSICAL PROPERTIES OF CANADIAN AND DANISH BACONS, AND THEIR RELATION TO FLAVOUR QUALITY¹

BY W. HAROLD WHITE², C. A. WINKLER³, AND W. H. COOK²

Abstract

Canadian bacon generally contained more curing salts, and was slightly drier and tougher than the Danish product. Although fat was present in approximately the same proportions in the bacons of both countries, that of Canadian bacon was slightly softer. The relative variability of Canadian and Danish bacons differed with the property considered. For Canadian bacon the most important source of the observed variations was usually the differences between the product prepared by different factories, whereas for Danish bacon, differences between sides were the principal source of variation.

Of the properties studied, flavour excellence in Wiltshire bacon was dependent primarily on the absence of excessive amounts of curing salts. Other contributing factors, of secondary importance, were high moisture content, tenderness, and a low proportion of good quality fat. Improvement in the flavour quality of Canadian Wiltshire bacon could be achieved by the use of milder cures, and the general adoption of improved handling and curing practices.

Introduction

The results of extensive investigations on the relative flavour excellence of Canadian and Danish Wiltshire bacons showed a superiority of the Danish over the Canadian product (9). It was impossible, however, to obtain reliable information from these subjective tests as to the causes of superiority or inferiority of a given product because of the difficulties involved in adequately describing quality, and the impossibility of suitably assessing the significance of such comments by statistical methods. Consequently, physical and chemical examinations of the bacons were made to determine the attributes of quality responsible for the superiority of the Danish product. The results of these objective measurements and their correlation with the subjective flavour scores are presented in this paper.

The selection of the measurements was based on those considered necessary to characterize the product, with particular emphasis being placed on the determinations that the comments of the tasters suggested as being important with respect to quality. The results of these analyses permitted a description of the product from the two countries with respect to the absolute values of the several properties and their variability. They also provided data that could be correlated with the subjective flavour scores obtained on the same

¹ Manuscript received April 18, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 65 of the Canadian Committee on Storage and Transport of Food, and as N.R.C. No. 998.

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material, thus giving information on the factors responsible for quality independent of the origin of the product.

Material and Methods

Since it was impossible to examine quantitatively all of the several thousand samples used in the flavour investigations, it was necessary to restrict the present studies to one group of samples. Of the material available, that from the incomplete randomized block experiment (9), in which each factory was represented by nine sides, was considered the most suitable for studying differences between and within factories. Furthermore, the results of this experiment provided reliable data for the computation of the relations between the measured properties and quality scores obtained from three independent panels.

Samples consisting of comparable portions of the prime back, directly posterior to the ribs, from nine sides selected from each of 18 Canadian and 10 Danish factories were packed in latex bags, sealed, boxed, and shipped at -12°C . (10°F .) from London, England. Upon arrival at Montreal, the boxes were packed in dry ice, and transferred immediately to the laboratories, where they were stored at -29°C . (-20°F .) until analysed. It is believed that as a result of these precautions little or no change in the measured properties occurred during shipment.

Of the several properties of bacon that might have been studied, the results of the flavour investigations suggested that the concentrations of chloride, nitrate, and nitrite, the content and quality of the fat, moisture content, and toughness would probably differ between the two countries, and correlate with the results of the flavour tests. In addition, these probably constitute the most important of the common properties of bacon, with the exception of colour.

Although all nine sides from each factory were used for the flavour tests, it was considered that fewer samples were required for the objective measurements described here, because of their greater accuracy and precision. The chloride, nitrate, and nitrite contents of five samples taken at random from the nine available for each of 18 Canadian and 10 Danish factories were determined by methods previously described (7).

The fat content was estimated by careful separation of the external layer from the lean meat, followed by weighing. Samples of three sides picked at random from each of 15 Canadian and nine Danish factories were treated in this manner.

Of the chemical methods available for estimating the quality of the fat, determination of the iodine number was considered to be most suitable (3, 5). Kaufmann's procedure (4) for this determination was used in the study of the external fat samples removed from three sides from each of 15 Canadian and nine Danish factories.

The moisture contents of five samples from each of 18 Canadian and 10 Danish factories were determined by drying *in vacuo* at 100° C. for 24 hr., or to constant weight.

Measurements of the relative toughness of samples of three sides from each of 15 Canadian and nine Danish factories were made by a method described previously (8).

Statistical methods (6) were used to reduce and interpret the data for each property. From the means, the standard deviation, and coefficient of variability between samples, it was possible to assess and compare the absolute and relative variability of the properties both within and between countries. An analysis of variance served to determine the relative importance of the possible sources of variation. The overall variability of Canadian and Danish Wiltshire bacons with respect to any one property may be divided broadly into differences in the properties of the sides within and between countries. Variations within countries may be further classified as differences between the sides produced in any one factory and differences between the product from different factories. For intercountry comparisons, differences between sides and between factories, as well as the overall difference between countries, can be distinguished. It was also of importance to assess the differences between factories and between sides within factories irrespective of country of origin.

The degree of interrelation of the properties studied with flavour score was estimated by the calculation of simple, and in some instances, partial, coefficients of correlation (6) between the mean values of the measurement and the quality score for each plant. The average values for factories rather than for countries were chosen for statistical treatment since they give a more representative evaluation of the properties studied. Although the use of the data for each side would have been still more suitable, the method of scoring for flavour, employed in the incomplete randomized block experiment, did not permit this. Correlation coefficients were calculated between the measurements and the flavour scores obtained from each of the three panels, since, although the over-all results for any one panel showed definitely that the quality of Canadian and Danish Wiltshire bacons differed, the relative order of the quality scores for plants within either country varied between the panels.

Results

Differences between Canadian and Danish Bacons

The mean values of the properties measured for Canadian and Danish Wiltshire bacons, together with analyses of variance are shown in Tables I to VII. Canadian bacon contained, on the average, significantly more sodium chloride, nitrate, and nitrite than Danish (Tables I, II, and III). The relative magnitude of these differences was especially large for nitrate, of which Canadian bacon contained approximately seven times more than the Danish samples studied. The fat of Canadian bacon also had a significantly

TABLE I

SODIUM CHLORIDE CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, %	7.47	6.21
Standard deviation, %	0.84	1.09
Coefficient of variability	11.3	17.5

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	50.8*
Between factories for both countries	26	7.31***
Between sides within factories for both countries	112	0.872
Between factories within Canada	17	9.66***
Between sides within Canada	72	0.698
Between factories within Denmark	9	2.87*
Between sides within Denmark	40	1.18

* Indicates 5% level of significance.

*** Indicates 0.1% level of significance.

TABLE II

SODIUM NITRATE CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, %	0.157	0.024
Standard deviation, %	0.028	0.015
Coefficient of variability	17.9	63.3

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	0.569**
Between factories for both countries	26	0.042***
Between sides within factories for both countries	112	0.0006
Between factories within Canada	17	0.0639***
Between sides within Canada	72	0.0008
Between factories within Denmark	9	0.0004
Between sides within Denmark	40	0.0002

** Indicates 1% level of significance.

*** Indicates 0.1% level of significance.

TABLE III
SODIUM NITRITE CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, p.p.m.	74	34
Standard deviation, p.p.m.	62	18
Coefficient of variability	85.1	51.3

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	49,504**
Between factories for both countries	26	6083**
Between sides within factories for both countries	112	2630
Between factories within Canada	17	8892**
Between sides within Canada	72	3921
Between factories within Denmark	9	778*
Between sides within Denmark	40	312

* Indicates 5% level of significance.

** Indicates 1% level of significance.

higher mean iodine number (Table VII). Bacon from both countries had approximately the same fat to lean ratio, namely 60 : 40 (Table VI). Although the average moisture content of Danish bacon was slightly higher, the difference failed to reach the level of statistical significance (Table IV).

TABLE IV
MOISTURE CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, %	59.6	61.1
Standard deviation, %	5.78	6.06
Coefficient of variability	9.69	9.93

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	133
Between factories for both countries	26	93.1***
Between sides within factories for both countries	112	34.6
Between factories within Canada	17	106**
Between sides within Canada	72	33.4
Between factories within Denmark	9	69.5*
Between sides within Denmark	40	36.8

* Indicates 5% level of significance.

** Indicates 1% level of significance.

*** Indicates 0.1% level of significance.

Canadian bacon was, on the whole, slightly tougher, but not significantly so, than the Danish product (Table V). The comments of the judges who made the subjective tests would suggest that definite differences in toughness did exist between Canadian and Danish bacons. That this was not confirmed here may be due to the fact that the objective measurements were made on uncooked material. It has been shown that toughness measurements should be made preferably on cooked samples (2).

TABLE V
TOUGHNESS OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, work units	2.40	2.09
Standard deviation, work units	1.13	0.903
Coefficient of variability	46.9	43.2

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	3.18
Between factories for both countries	22	1.53
Between sides within factories for both countries	48	0.908
Between factories within Canada	14	1.83
Between sides within Canada	30	1.27**1
Between factories within Denmark	8	0.985
Between sides within Denmark	18	0.816**1

** Indicates 1% level of significance.

¹ Exceeds 1% level of significance when compared with the mean square attributable to experimental error.

Differences in the properties of sides from different factories regardless of country of origin significantly exceeded variations in sides within the same factories for the sodium chloride, nitrate, nitrite, and moisture contents. This suggests that these properties are to some extent a function of factory practice. Similar comparisons for toughness (Table V), fat content (Table VI), and the iodine number of the fat (Table VII) were not significant.

It was also of interest to determine the major source of variability in the sides from each of the two countries. Such a comparison for Canadian bacon showed that differences between sides originating from different factories significantly exceeded variations within these same factories for the sodium chloride, nitrate, and nitrite contents. Similar comparisons for Danish bacon showed significant differences in the sodium chloride and nitrite contents. Both Canadian and Danish sides showed significant variations in toughness, indicating that tenderness, as determined here, was an inherent property of the side.

TABLE VI
FAT CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean, %	58.9	59.5
Standard deviation, %	6.06	6.13
Coefficient of variability	10.3	10.3

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	6.22
Between factories for both countries	22	34.4
Between sides within factories for both countries	48	37.0
Between factories within Canada	14	37.7
Between sides within Canada	30	36.7
Between factories within Denmark	8	28.6
Between sides within Denmark	18	37.6

TABLE VII
IODINE NUMBER OF FAT OF CANADIAN AND DANISH WILTSHIRE BACONS

Statistic	Country	
	Canada	Denmark
Mean	55.6	52.1
Standard deviation	4.04	3.76
Coefficient of variability	7.26	7.23

ANALYSIS OF VARIANCE

Variance attributable to:	D.f.	Mean square
Between countries	1	214**
Between factories for both countries	22	11.7
Between sides within factories for both countries	48	15.5
Between factories within Canada	14	17.1
Between sides within Canada	30	16.3
Between factories within Denmark	8	2.34
Between sides within Denmark	18	14.2

**** Indicates 1% level of significance.**

Although similar treatments for the other properties failed to reach the level of statistical significance, it is of interest to note that for Canadian bacon the variance attributable to differences between factories was usually greater than that for differences between sides within factories, whereas for the Danish product the more important source of variance was between sides.

Interrelation of Objective Measurements with Flavour Scores

The values for the coefficients of correlation between the content of curing salts and quality score are shown in Table VIII. The concentration of sodium

TABLE VIII

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE, NITRATE, AND NITRITE CONTENT OF CANADIAN AND DANISH WILTSHIRE BACONS AND FLAVOUR SCORE

Quantities correlated	Smithfield jury test		Importers' jury test		Family group test	
	D.f.	<i>r</i>	D.f.	<i>r</i>	D.f.	<i>r</i>
Flavour score with:						
Content of sodium chloride	26	-0.61**	26	-0.47*	26	-0.51**
Content of sodium nitrate	26	-0.27	26	-0.39*	26	-0.44*
Content of sodium nitrite	26	-0.31	26	-0.40*	26	-0.34
Total salt content	26	-0.61**	26	-0.49**	26	-0.53**
Content of sodium nitrate independent of sodium chloride	25	-0.12	25	-0.30	25	-0.36
Content of sodium nitrite independent of sodium chloride	25	+0.03	25	-0.20	25	-0.09

* Indicates 5% level of significance.

** Indicates 1% level of significance.

chloride was significantly and negatively related to the score for all three panels. The correlations with nitrate were negative for each, and reached the level of statistical significance for the Importers' and family group panels. Nitrite was also negatively related to quality score for all three panels, but significantly so only for the Importers' panel. Since negative relations were obtained in all instances, it is implied that the eating quality is adversely affected when excessive quantities of the curing salts are present. The fact that the relations observed for nitrate or nitrite were significant for one panel and not for another may be merely fortuitous, or may be a reflection of the ability of a particular panel to distinguish between the flavour imparted by the three salts.

To determine the effect of concentration of nitrate or nitrite on flavour quality independently of chloride, which was present in much greater concentration, partial coefficients of correlation were calculated. These were negative in most instances, but failed to reach the level of statistical significance. This suggested that the most important factor was the quantity, rather than the kind, of curing salt. To test this, simple coefficients of correlation were calculated between quality score and total concentration of

salts, i.e., sum of nitrate, nitrite, and chloride. These were negative, highly significant, and, in two instances, greater than those obtained for chloride alone, although not significantly so. This indicates that the flavour quality decreased if excessive amounts of curing salts were present, regardless of the nature of the salt. However, there is some indication that chloride, present in much larger quantities, may mask the effect of nitrate and nitrite on eating quality.

Since, as has been shown previously, the mean concentrations of all three salts were greater in Canadian than in Danish bacon, the quality of Canadian bacon could be improved by the use of milder cures, in conjunction with suitable supplementary methods of preservation if necessary.

TABLE IX

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN MOISTURE CONTENT AND TOUGHNESS OF CANADIAN AND DANISH WILTSHIRE BACONS AND FLAVOUR SCORE

Quantities correlated	Smithfield jury test		Importers' jury test		Family group test	
	D.f.	<i>r</i>	D.f.	<i>r</i>	D.f.	<i>r</i>
Flavour score with:						
Per cent moisture calculated on basis of:						
Whole meat	26	0.30	26	0.43*	26	0.42*
Salt-free meat	26	0.04	26	0.24	26	0.21
Toughness	22	-0.20	22	0.26	22	-0.19

* Indicates 5% level of significance.

Simple coefficients of correlation between moisture content, as calculated on a whole meat basis and quality score, were significant and positive for the Importers' panel and family group tests (Table IX). This implies that good quality in bacon is associated with a high moisture content. However, since the moisture content is to some extent dependent on the concentrations of salts present, the observed relations might merely be a reflection of the effect of salt concentration. This was tested by calculating the moisture contents on a salt-free basis, and subsequently correlating these values with the quality scores. These values were positive for each panel, but failed to reach the level of statistical significance. This would suggest that there was no relation between the moisture content of the bacon and its quality as determined here.

The coefficients obtained in comparisons of toughness with quality score were in most instances negative, but not statistically significant (Table IX). As would be expected, toughness adversely affected quality, but was apparently of less importance than the content of curing salts.

The correlations between quality score and the measurements made on the fat are shown in Table X. The coefficients for the quality of the fat, as

estimated by the iodine number, were for the most part negative, but statistically significant for the Smithfield panel alone. This indicates that the over-all flavour quality of Wiltshire bacon decreased with an increase in iodine number, i.e., a decrease in fat quality. It may be that the more unsaturated the fat, the less stable it is during cooking, with the consequent enhanced formation of products unpleasant to the taste.

TABLE X

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN CONTENT AND IODINE NUMBER OF FAT OF CANADIAN AND DANISH WILTSHIRE BACONS AND FLAVOUR SCORE

Quantities correlated	Smithfield jury test		Importers' jury test		Family group test	
	D.f.	<i>r</i>	D.f.	<i>r</i>	D.f.	<i>r</i>
Flavour score with:						
Content of fat	22	-0.01	22	0.22	22	-0.14
Iodine number of fat	22	-0.49*	22	0.05	22	-0.14

* Indicates 5% level of significance.

The content of fat was for the most part negatively, but not significantly, related to the quality score. This would imply that an excess amount of fat is detrimental to quality.

Discussion

Of the physical and chemical properties of Wiltshire bacon considered here as bearing a possible relation to quality, the content of curing salts was the most important. Although the observed variations cannot be attributed entirely to this factor alone, it must be recognized that the quality of Canadian Wiltshire bacon could be improved by the use of milder cures.

As a whole the data show that the quality of the fat of Canadian Wiltshire bacon sides is satisfactory (3). However, in some instances the fat would be classed as definitely soft. In addition, it is evident that a more uniform selection and grading of sides both within and between Canadian factories exporting Wiltshire bacon would be desirable.

However, with the exception of the content of curing salts, Canadian bacon compares favourably with Danish for the properties studied here. In most instances the bacon of Canadian manufacture was more variable in nature than that originating from Denmark. For Canadian bacon the most important source of the observed variations was between sides originating from different factories. The major source in the Danish product lay between the individual sides. This suggests that considerable uniformity in factory and curing practices exists in Denmark, whereas in Canada, although each factory prepares a relatively uniform product, undesirable variability exists between the various companies exporting Wiltshire bacon. This is in agreement with the results of the subjective tests on flavour quality.

The relative variability of Canadian and Danish factories for each of the properties studied here can be seen readily from the series of histograms shown in Fig. 1. In their preparation the class interval was made approximately equal to the necessary difference for statistical significance computed for the variance between sides within factories irrespective of the country of origin (1). In this way the observed mean values for factories are distributed over the number of classes that can be distinguished experimentally from one another. It is to be noted, however, that individual results in adjacent classes may not differ significantly. Since these are self-explanatory, further discussion of them is unnecessary.

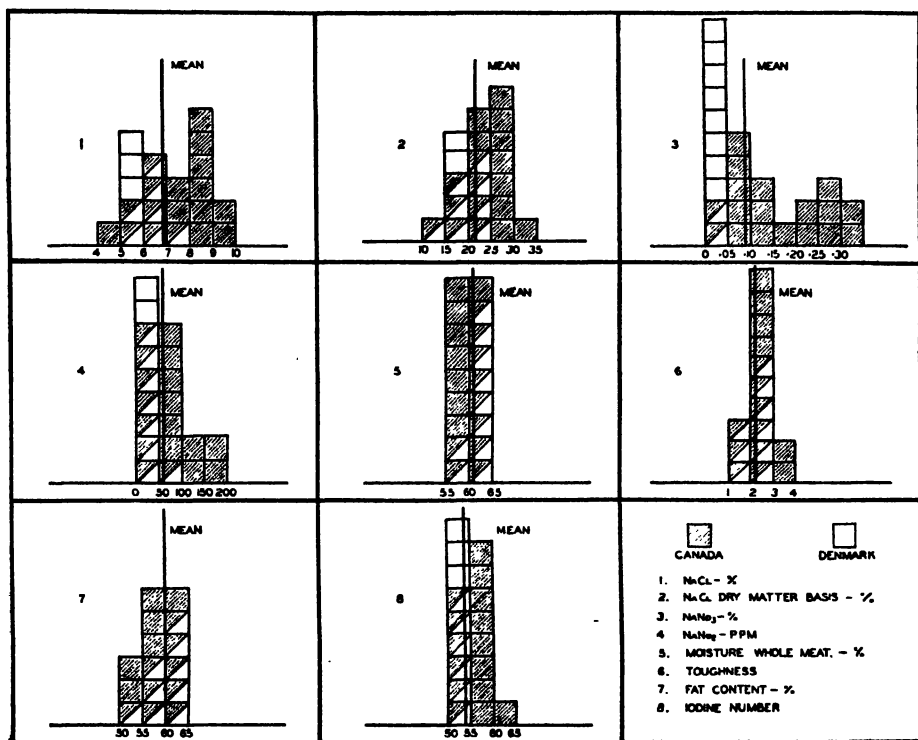


FIG. 1. Histograms for the measured properties of Canadian and Danish Wiltshire bacons.

The concentrations of chloride in the samples studied here were much higher than those previously obtained in 1938 for Canadian Wiltshire bacon (1). At that time it was found that the mean chloride content on a moisture- and salt-free basis was 18.2%, whereas here the mean for the same factories on a similar basis was 22.7%. These differences were found to be statistically significant.

The observed increase is presumably due either to the use of stronger curing solutions or to differences in sampling position studied in the two years. The samples examined in 1938 were from the ham, whereas those in 1939 were

removed from the back. Although it has been shown previously that significant differences with position exist even within one distinct portion of the side (1), it is doubtful if the magnitude of these normal variations in concentration is sufficiently great to account for the differences observed here.

Since significant difference in the concentrations of chloride existed between the two years, it is to be expected that the moisture content would also differ. It was found that the mean moisture content, calculated on a salt-free basis, of the samples studied here was approximately 65% as compared to a value of 73%, obtained in 1938, for samples from the same factories, which were stored and smoked on an experimental basis. Since the 1938 samples consisted of small cuts and were probably exposed to an atmosphere of lower relative humidity prior to analysis, the moisture content of these might be expected to be lower than those of 1939. An explanation of this apparent anomaly may be that British smoking practices cause excessive drying of the sides. This may possibly account for the criticism of "hardness" sometimes levelled at Canadian bacon.

Acknowledgments

The authors wish to express their appreciation to Messrs. E. G. Blake and E. A. Rooke, Laboratory Assistants, and to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for their able assistance.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

AUGUST, 1941

NUMBER 8

CANADIAN WILTSHIRE BACON

XXI. OBJECTIVE COLOUR COMPARISONS OF CANADIAN AND DANISH BACONS, AND THEIR RELATION TO SUBJECTIVE OBSERVATIONS OF COLOUR QUALITY¹

BY C. A. WINKLER², G. ABEL³, AND W. H. COOK⁴

Abstract

Both smoked and unsmoked Danish bacons were lighter in colour than Canadian, as determined by objective measurements with a photoelectric comparator. Little difference existed in the colour stability of the product from the two countries.

Comparison of the objective measurements with subjective estimates of colour quality indicates that for the colour comparator employed, bacons having scatter values of 30 to 36% in the red, 16 to 21% in the green, 13 to 17% in the blue and a total of 58 to 74% relative to a white standard, would be considered to have a good colour.

Introduction

The relations between colour and colour stability of Canadian Wiltshire bacon and certain aspects of curing practice, postcuring treatment, and chemical composition have been dealt with in previous communications from these laboratories (4, 5, 6, 7). In these studies a photoelectric colour comparator, described in detail elsewhere (3), was used to obtain an objective measure of colour and colour stability. Data obtained by this method, while essential and satisfactory for investigational purposes, could obviously give no information about consumer preference for colour. It was therefore impossible to assess the true significance of the numerical values used to express colour and colour stability. If, however, the objective measurements could be related to subjective observations of the consumer, a basis could be established on which to estimate the relative colour excellence of different bacons, and to determine whether or not changes in colour, from whatever cause, were to be regarded as deleterious or beneficial.

As with the flavour studies reported previously (2) a reliable consumer opinion of colour could be obtained only from representatives of the English

¹ Manuscript received April 28, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 66 of the Canadian Committee on the Storage and Transport of Food, and as N.R.C. No. 1001.

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public, since practically all the Wiltshire bacon of Canadian manufacture is consumed in England. An improved type of photoelectric colour comparator (3) was therefore taken to England at the time of the flavour investigations and used, not only to establish the relation between consumer observations and objective measurements, but also to compare the colour and colour stability of Canadian bacon with that of Danish bacon, which may be regarded as a standard of quality on the English market. The results of both these colour studies are included in the present paper.

Experimental

The materials used for the colour investigations were portions of the Danish and Canadian bacons obtained from wholesale distributors in London and submitted to the jury groups during flavour tests described previously (2). Both smoked and unsmoked bacons, taken from 10 Danish and 10 Canadian factories selected at random, were represented. With minor exceptions two sides each of smoked and unsmoked bacon were taken to represent each factory.

The method of making colour and colour stability measurements with the photoelectric comparator has been described in an earlier paper (1) and need not be discussed here.

The method of treating the data statistically has also been discussed in detail elsewhere (4, 6, 7).

The subjective observations were made by the six members of each jury group responsible for the flavour tests on corresponding samples of the same materials. Each member worked quite independently of others, and judged the colour of each sample on two successive days. The observations were in the form of comments, however, and are best treated as if the 12 comments per sample were entirely independent judgments.

Results

OBJECTIVE COLOUR COMPARISONS OF CANADIAN AND DANISH BACONS

Internal Colour

A complete analysis of variance is given in Table I for the colours of both smoked and unsmoked bacons. The error includes the average difference between first and second sides from the same factories (days 1 to 10 versus days 11 to 19), since these differences were not significant. From the table it is seen that a significant difference existed between the colours of unsmoked bacons of Danish and Canadian origin, but this difference between countries was not evident for the smoked product. For neither smoked nor unsmoked material was there any significant difference between factories of a given country. The means by countries for both classes of bacon over the entire test, presented in Table II, show that the Danish samples were brighter than the Canadian samples in all colour components, although as shown in Table I this difference only attains significance for the unsmoked materials.

TABLE I
INTERNAL COLOUR OF DANISH AND CANADIAN BACONS
ANALYSIS OF VARIANCE

Variance due to:	D.f.	Scatter			
		Mean square			
		Blue	Green	Red	Total
Unsmoked					
Between countries	1	25.4*	75.1**	103.0**	932.0**
Between factories (Can.)	9	5.46	9.72	17.4	88.3
Between factories (Dan.)	9	3.38	6.48	5.41	44.9
Error	19	3.73	8.05	12.2	69.3
Smoked					
Between countries	1	10.6	29.4	20.5	178.0
Between factories (Can.)	9	2.69	4.77	13.9	55.3
Between factories (Dan.)	9	3.59	7.08	14.1	65.5
Error	17	3.91	7.47	8.73	56.5

* Indicates 5% level of significance.

** Indicates 1% level of significance.

TABLE II
INTERNAL COLOUR OF DANISH AND CANADIAN BACONS

Country	Mean scatter, %			
	Blue	Green	Red	Total
Unsmoked				
Canadian	14.0	17.5	31.4	62.9
Danish	15.6	20.2	34.7	70.3
Smoked				
Canadian	15.7	19.7	34.7	70.1
Danish	16.8	22.5	36.2	74.5

Colour Stability

The analysis of variance given in Table III shows that the only significant differences in colour stability lie between the repetitions of the experiment (i.e., between days 1 to 10 and days 11 to 19), or the average difference between sides from the same factory. The values only attain significance for blue and green in unsmoked bacon and for green in smoked bacon. This suggests that colour stability is a function of the individual side, although this cannot be stated with certainty.

TABLE III
COLOUR STABILITY OF DANISH AND CANADIAN BACONS
ANALYSIS OF VARIANCE

Variance due to:	D.f.	Mean square			
		Blue	Green	Red	Total
Unsmoked					
Between countries	1	0.02	1.26	17.3	9.08
Between factories (Can.)	9	0.97	1.48	4.63	10.5
Between factories (Dan.)	9	0.63	0.88	4.27	9.82
Between sides within factories	0 or 1	7.79**	16.8**	—	—
Error	18 or 19	0.72	0.76	8.68	13.8
Smoked					
Between countries	1	0.46	1.85	1.53	1.93
Between factories (Can.)	9	0.72	0.63	2.09	3.85
Between factories (Dan.)	9	0.55	0.98	1.95	5.31
Between sides within factories	0 or 1	—	4.52*	—	—
Error	16 or 17	0.51	0.74	2.22	7.80

* Indicates 5% level of significance.

** Indicates 1% level of significance.

TABLE IV
COLOUR STABILITY OF DANISH AND CANADIAN BACONS

Country	Change in scatter, %				
	Test	Blue	Green	Red	Total
Unsmoked					
Canadian	1 - 10	0.56	0.02	2.70	3.40
	11 - 19	-0.33	-1.21	3.64	1.94
Danish	1 - 10	0.53	0.49	1.71	2.73
	11 - 19	-0.39	-0.77	1.92	0.76
Smoked					
Canadian	1 - 10	-0.26	-0.86	1.24	0.35
	11 - 19	0.58	-0.97	1.16	0.91
Danish	1 - 10	0.12	0.14	0.67	0.93
	11 - 19	-0.53	-1.02	0.90	-0.65

Table IV shows the mean change in colour for Danish and Canadian bacons for both test periods and both classes of bacon. The consistently larger increases or smaller decreases for the samples tested on days 11 to 19 as compared with the changes during days 1 to 10 might suggest that the storage conditions during the two test periods were somewhat different. If this were true the significant differences noted between sides within factories might not present a true picture. Experimental conditions were not, however, appreciably or consistently different during the two test periods. In any case, there is little evidence that Canadian bacon is inferior to Danish in colour stability.

RELATIONS BETWEEN SUBJECTIVE AND OBJECTIVE OBSERVATIONS

Owing to certain omissions, the results of the subjective observations on colour consisted of 438 comments on unsmoked material and 430 comments on smoked material. These comments were divided into six classes, viz.: good, pale, dark, fair, poor, and miscellaneous, the last class including all comments that could not be placed in the first five.

To relate the subjective observations to the four independent objective colour measurements without making arbitrary assumptions, the following procedure was adopted.

The range of objective values for, say, blue was divided into five classes. A given sample could now be placed in a given objective class and the comments of different judges on that sample distributed among the subjective categories. In this way the 38 objective measurements on smoked bacon were divided into five classes, each of which included six subjective categories.

This procedure was then repeated for the green and red measurements. Naturally, there were the same number and distribution of comments in each subjective category, but the objective measurements to which these were to be related were different for each colour. It was hoped, therefore, that this procedure might make it possible to determine the objective measurement in best agreement with the judges' comments.

This procedure reduced the results to the form of frequency diagrams (Fig. 1) having numerical values for each class capable of statistical treatment. To provide a test of significance, the subjective and objective totals for each objective class were computed. From these totals it was possible to compute the number to be expected in each subjective class, i.e., good, pale, etc., if the judges had no discriminating power and merely placed the samples in the various objective classes within subjective categories in accordance with the distribution of the totals over all the objective classes.

Having thus obtained "Observed" and "Expected" values in each row and column, it was possible by means of the Chi square test to determine whether or not the "Observed" value differed significantly from the "Expected" value. The values obtained for Chi square are given in Table V and are significant in all cases. This shows that the judges were, on the average,

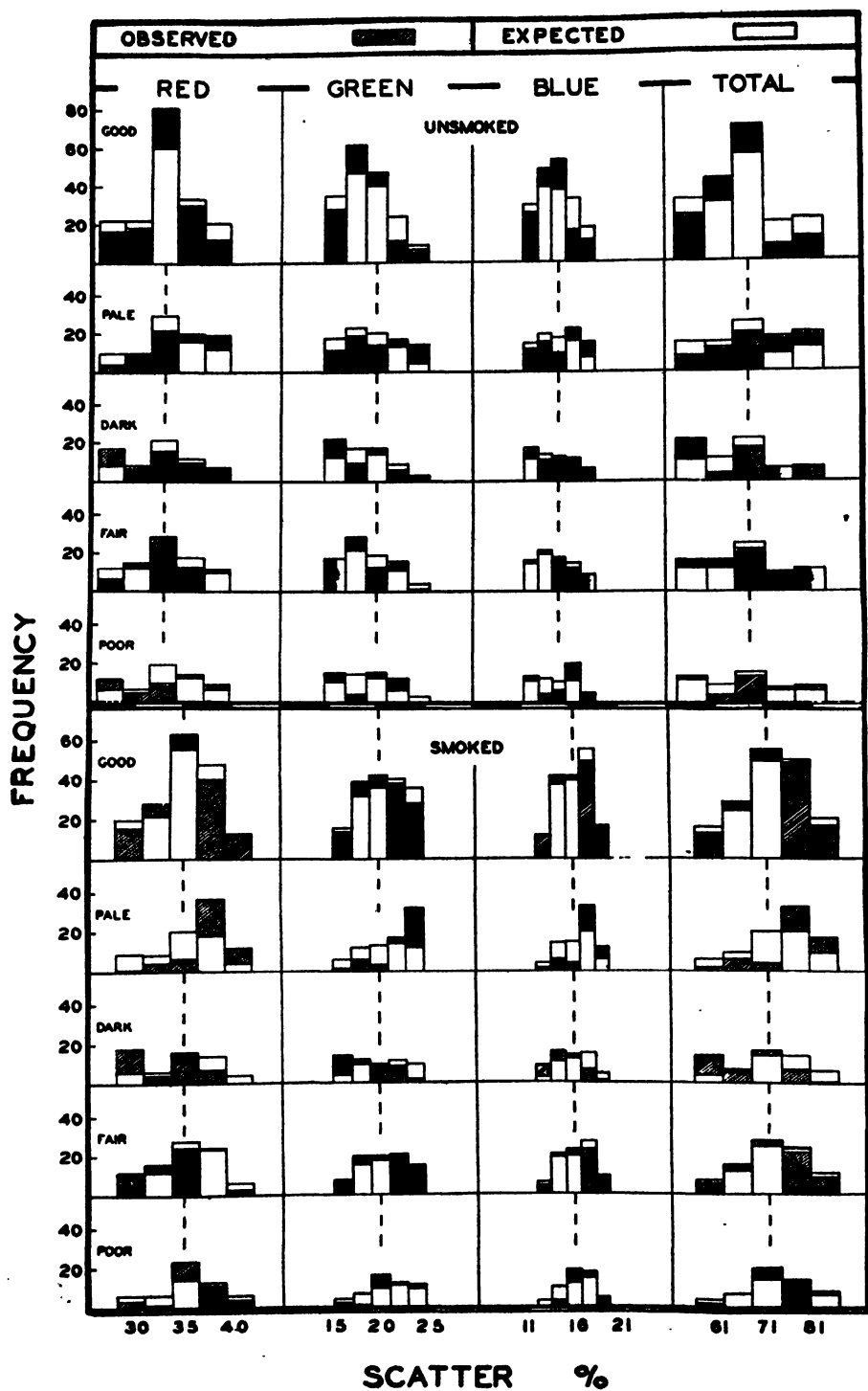


FIG. 1. Frequency diagrams relating subjective and objective estimates of colour.

capable of discriminating between the different colour classes adduced from the instrumental readings.

This method of analysis tests the significance of the differences for the block of data as a whole, and more detailed information must be secured from examinations of the original results and computed values.

TABLE V
VALUES OF CHI SQUARE OBTAINED IN TEST OF SIGNIFICANCE OF
DIFFERENCES OF FREQUENCY OF SUBJECTIVE OBSERVATIONS
FOR EACH OBJECTIVE COLOUR CLASS

Type of bacon	Objective colour class			
	Blue	Green	Red	Total
Unsmoked	67.5**	86.4**	56.9**	68.9**
Smoked	76.4**	94.6**	104.0**	85.0**

NOTE: Value of Chi square required for 1% level of significance with 20 degrees of freedom = 37.6.

** Indicates 1% level of significance.

The essential information arising from such an examination has been represented in Fig. 1. It shows the "Observed" and "Expected" values from which the reader can gain some idea of the magnitude of the difference between the two values for each independent class he may wish to compare. The frequency for both of these classes is measured from the base line so that the higher value is evident from examination. Since the red, green, blue, and total colour scatters were somewhat lower for the unsmoked than for the smoked product, the difference between them is shown in the correct relation by plotting the results from both types of bacon on the same abscissa scale.

Although Table V shows that the differences between the "Observed" and the "Expected" values are significant over all subjective observations in a given colour band, no test of significance is available for the individual objective colour class in each subjective category. Where the "Observed" value exceeds the "Expected" by a reasonable margin, however, it may be concluded that these classes contributed to the observed differences and that these objective classes are typical of the category in which they were placed by the judges.

Referring to the measurement of red in the unsmoked bacon, in the central class the number of comments in the "Good" category is in excess of the average expectation, whereas in the two end classes it is below expectation. This indicates that bacon having red scatters in the central range, i.e., 31 to 34, is most likely to be considered "Good". This conclusion is supported by the fact that the "Observed" exceeded the "Expected" in the two highest classes in the "Pale" category, indicating that samples having these red scatters, i.e., 34 to 40, are most likely to be considered "Pale". The results

are not so definite for the "Dark" class but generally speaking, samples between 25 and 30 would be considered "Dark". Since samples classified as "Fair" or "Poor" may be either "Pale" or "Dark", no definite conclusion can be drawn from these categories, but these histograms are included; those for the "Miscellaneous" have been omitted. It should be noted, however, that the "Expected" values were, in all cases, computed from the total observations over all categories, including those in the "Fair", "Poor", and "Miscellaneous" groups. Doubtless the values of Chi square in Table V would have been even larger if these classes had been excluded, but since the values are already overwhelmingly significant, no advantage would have been gained from such a procedure.

TABLE VI
REFLECTIVITY OF BACONS JUDGED TO HAVE "Good" COLOUR

Sample	Colour scatter in terms of white standard, %			
	Red	Green	Blue	Total
Unsmoked	31 - 34	16 - 21	13 - 16	58 - 71
Smoked	30 - 36	17 - 21	13 - 17	61 - 74
Most probable desired values	30 - 36	16 - 21	13 - 17	58 - 74

By an extension of the analyses indicated above, for all colours, for both unsmoked and smoked bacon, the values listed in Table VI are obtained as the most likely reflectivity of samples judged to have "Good" colour. Naturally, the decisions reached as to the best colour are, to some extent, arbitrary, but they do provide a working basis for future investigations.

Acknowledgment

The authors wish to express their grateful thanks to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for assistance with the statistical computations.

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CANADIAN WILTSHIRE BACON

XXII. COMPARATIVE CARCASS QUALITY OF CANADIAN AND DANISH BACONS¹

By C. A. WINKLER², W. HAROLD WHITE³, AND G. ABEL⁴

Abstract

The opinions of expert judges indicated that Canadian and Danish bacon carcasses, as received on the English market, were, in general, of approximately the same quality. The eye of lean of Canadian sides was on the average slightly longer, but of approximately the same width as that of Danish carcasses. In the Canadian product the variations in the size of the eye of lean appeared to be a property of the individual side, whereas the Danish sides showed significant variations between factories.

Computations of the degree of interrelation of the measured properties demonstrated that good quality in a bacon carcass varied directly with the size of the eye of lean, and indirectly with the fat content. An increase in the content of fat was associated with a decrease in the size of the eye of lean.

Introduction

It has been shown in previous papers that definite differences in flavour quality (8) and in a number of physical and chemical properties existed between Canadian and Danish Wiltshire bacons (6, 7). It was also found that certain of the properties studied, such as the content of curing salts and quality of the fat, were related to the over-all flavour quality of the bacon side (6). However, it is evident that, irrespective of the suitability of the procedure employed for the conversion of pork to bacon, the quality of the final product is to a large extent dependent on that of the original hog carcass.

The carcass characters of the best commercial Danish bacon pig and the best breeding stock in English Large White breed have been compared by McMeekan (2). The purpose of the present paper is to present certain observations made on the comparative carcass quality of commercial Canadian and Danish Wiltshire bacon sides.

Material and Methods

The samples studied in this investigation consisted of sections of the side, approximately 2 to 3 in. in thickness, removed immediately behind the ribs and extending through the prime back. Such samples were obtained for nine sides picked at random from each of 18 Canadian and 10 Danish factories

¹ Manuscript received May 29, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as Paper No. 68 of the Canadian Committee on the Storage and Transport of Food, and as N.R.C. No. 1005.

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exporting Wiltshire bacon to England. After the grading and measurement of the eye of lean, the samples were frozen and shipped to the National Research Laboratories, as described previously, where the proportion of the fat was determined (6).

Each of the samples was graded by a committee consisting of three men of long experience in the Wiltshire bacon trade. As a result of a general inspection of the samples they considered that four definite classes could be distinguished, to one of which each sample was subsequently allocated. In addition to conformation, such factors as poor singeing and butchering and softness of the fat were considered.

Physical measurements of the eye of lean (longissimus dorsi muscle) have been used by many investigators as an index of carcass quality (1, 2, 3, 5). An approximate measure of the cross sectional area of this muscle was obtained here as the product of its maximum length and width (5).

It was also of importance to determine the proportion of physically separable fat present in the samples. The fat was carefully removed with a knife, and was then weighed.

Results

Statistical methods were employed to reduce and interpret the data (4). The relative importance of the sources of variations was determined by means of an analysis of variance. Possible sources considered here include differences between carcasses from the same factory and differences between carcasses from different factories in either Denmark or Canada, and the over-all difference between countries. Differences noted here for within factories may be considered to represent variations in carcasses from a local district or

TABLE I
CLASS OF CANADIAN AND DANISH WILTSHIRE BACON SIDES

Statistic	Country	
	Canada	Denmark
Mean	2.33	2.21
Standard deviation	0.93	0.74
Coefficient of variability	40.0	33.3

Analysis of variance		
Variance attributable to:	D.f.	Mean square
Between Canadian factories	17	1.30
Within Canadian factories	138	0.816
Between Danish factories	9	0.856
Within Danish factories	66	0.499
Between countries	1	0.770
Within countries	230	0.762

variations in uniformity of grading within the individual factories. Differences between factories may be due to variations in carcasses over a wider area, or to differences in grading in the various factories. The degree of interrelation of the properties studied was estimated by the calculation of simple coefficients of correlation, and in certain instances by means of an analysis of variance and correlation ratios.

Although the mean class assigned to both Danish and Canadian Wiltshire bacon sides was approximately two, Canadian sides were of a slightly poorer grade and more variable in nature (Table I). There was some indication that variations in grading standards existed between factories both in Denmark and Canada, but such differences failed to reach the level of statistical significance. The differences between countries were likewise not significant. While the class of side commercially employed for conversion to Wiltshire bacon was better for Denmark than for Canada, there is some indication that Canadian Wiltshire sides are of a satisfactory quality.

Since it is apparent that the product of the length and width gives only an approximate estimation of the area of the eye of lean, the statistical computations were made for each of the measurements. The results of the analyses of variance, together with the mean values are shown in Table II.

TABLE II

LENGTH, WIDTH, AND AREA OF THE EYE OF LEAN OF CANADIAN AND DANISH WILTSHIRE BACON SIDES

Statistic	Measurement of the eye of lean					
	Length, cm.		Width, cm.		Area, sq. cm.	
	Canada	Denmark	Canada	Denmark	Canada	Denmark
Mean	9.08	8.90	2.82	2.84	25.7	25.4
Standard deviation	0.78	0.62	0.42	0.45	4.93	4.85
Coefficient of variability	8.59	6.96	14.7	15.8	19.2	19.1

Analysis of variance						
Variance attributable to:	Measurement of the eye of lean					
	Length		Width		Area	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Between Canadian factories	17	0.496	17	0.252	17	26.3
Within Canadian factories	134	0.623	134	0.162	134	24.1
Between Danish factories	9	0.618	9	0.644**	9	74.5**
Within Danish factories	59	0.348	59	0.133	59	15.7
Between countries	1	1.44	1	0.016	1	5.03
Within countries	219	0.539	219	0.181	219	24.1

* Indicates 1% level of significance.

The eye of lean of Canadian Wiltshire sides was on the average slightly longer, but of approximately the same width as those of Danish bacon sides. The results obtained here for commercial Danish sides were slightly greater for the length and less for the width of the eye of lean than those reported previously for the best Danish commercial product and English breeding stock in the Large White breed (2).

From the analysis of variance it may be seen that, for any of the three measurements, the average differences between sides from different Canadian factories did not exceed those attributable to sides within these factories. However, variations between the width and area of the eye of lean of the product from Danish factories significantly exceeded the differences within factories. Moreover, the variability between Danish factories in the width and area of the eye of lean was significantly greater than that between Canadian factories. In no instance, did the over-all difference between countries exceed that within countries. While the most important differences in the properties of the eye of lean for Canadian bacon occurred between sides, the major source of variance for Danish bacon was between factories.

TABLE III

INTERRELATION OF CLASS AND MEASUREMENTS OF THE EYE OF LEAN OF CANADIAN AND DANISH WILTSHIRE BACON SIDES

Class	Length, cm.		Width, cm.		Area, sq. cm.	
	Mean	Necessary difference ¹	Mean	Necessary difference	Mean	Necessary difference
1	9.54		3.15		30.2	
2	9.00	0.345	2.89	0.19	26.1	2.13
3	8.81	0.313	2.62	0.17	23.0	1.93
4	8.76	0.461	2.51	0.26	22.1	2.84

Analysis of variance

Variance attributable to:	Length		Width		Area	
	D.f.	Mean square	D.f.	Mean square	D.f.	Mean square
Between classes	3	4.85**	3	3.05**	3	496**
Within classes	217	0.460	218	0.140	217	17.5
Correlation ratio of class with:	0.357**		0.480**		0.531**	

¹ Necessary difference between adjacent classes for 5% level of significance.

** Indicates 1% level of significance.

The results of the determinations on fat content have been discussed in a previous paper (6). In brief, it was found that sides from both countries had an approximate fat to lean ratio of 60 : 40. There was some indication that differences between factories were most important for Canadian Wiltshire bacon, whereas in Denmark the prime factor was variations between sides.

Having assessed the significance of the possible sources of variation for the properties studied here, it was of importance to ascertain the extent to which these were interrelated independent of country of origin. The relation of class to length, width, and area of the eye of lean was determined by means of an analysis of variance, which is presented, together with class means, in Table III. Since the differences between classes of sides significantly exceeded those within classes for all measurements of the eye of lean, any one of the three measurements of the eye of lean is related to the over-all quality of Wiltshire bacon sides. A quantitative estimate of the interrelation, obtained by the computation of correlation ratios for each of the properties, indicated that the area, as determined here, was most closely related to the class of side.

While the over-all differences between classes were highly significant, those between adjacent grades were significant only for the first three classes. This would suggest that on the basis of the eye of lean, the judges were able to distinguish only three classes. Such factors as poor butchering, excessive singeing, poor quality of the fat, etc., may possibly have been responsible for the assignment of a side to the fourth grade.

The results of an analysis of variance for differences in fat content with class of Wiltshire bacon side showed that the over-all variations between classes exceeded those within classes (Table IV). However, when the mean fat content was considered by classes, the differences between adjacent classes

TABLE IV

INTERRELATION OF CLASS AND CONTENT OF FAT OF CANADIAN AND DANISH WILTSHIRE BACON SIDES

Statistic	Class			
	1	2	3	4
Mean	56.1	57.4	61.9	63.6
Necessary difference ¹	5.5	4.6	5.8	

Analysis of variance		
Variance attributable to:	D.f.	Mean square
Between classes	3	178**
Within classes	63	29.4
Correlation ratio of class with fat content	0.473**	

¹ Necessary difference between adjacent classes for 5% level of significance.

** Indicates 1% level of significance.

failed to reach the level of statistical significance. The significant correlation ratio between class and fat content showed that poor quality in the side was associated with a high content of fat.

Simple coefficients of correlation between the content of fat and the measurements of the eye of lean were in each instance negative, and reached the level of statistical significance for the width and area (Table V). This suggests that an increase in the content of fat was associated with a decrease in the size of the eye of lean.

TABLE V

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE OBSERVED PROPERTIES OF CANADIAN AND DANISH WILTSHIRE BACON SIDES

Quantities correlated	D.f.	
Content of fat with:		
Class	65	.46**
Length of eye of lean	63	— .22
Width of eye of lean	63	— .54**
Area of eye of lean	63	— .52**
Class with flavour score:		
Importers' jury group	26	.07
Smithfield jury group	26	— .19
Family group test	26	— .17
Area of eye of lean with flavour score:		
Importers' jury group	26	.07
Smithfield jury group	26	— .08
Family group test	26	— .10

** Indicates 1% level of significance.

It was of interest to determine the extent of correlation between the subjective estimates of eating quality, as provided by the jury groups and family group flavour tests (8), and those of carcass quality of the Wiltshire bacon side. The correlations between class and area of the eye of lean and flavour score were for the most part negative, but not statistically significant. However, since the majority of these were negative, there is some indication that quality in the Wiltshire carcass is reflected in the eating quality of the bacon.

Discussion

From the results presented here it would appear that the average Canadian Wiltshire side compares favourably with that of Denmark.

In addition to grading, the judging committee commented on excess singeing of the bacon sides. Of the Canadian and Danish sides, 14 and 23%, respectively, were criticized for defective singeing. Canadian factories would appear to be definitely superior with respect to precautionary measures taken to minimize scorching of the sides. Complaints of scorching of the Canadian product, however, were made against seven sides treated in vertical types

of singers, nine in the Danish "clam-shell" type, and two prepared by each of the hand, open, and horizontal methods. The proportions of complaints, when considered with respect to factories, were approximately the same for all the types of singeing commonly employed in Canadian practice.

Acknowledgments

Grateful acknowledgment is made to Dr. J. W. Hopkins, Statistician, National Research Laboratories, for his advice and assistance with the statistical computations, and to the members of the grading committee for their graciously given time and talent.

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THE CULTIVATION OF TAPEWORMS IN ARTIFICIAL MEDIA¹

BY NANCY KATHARINE GREEN² AND ROBERT ARNOLD WARDLE³

Abstract

Experiments were made with the tapeworms *Diphyllbothrium latum* (L.), *Moniezia expansa* (Rud.), and *Hymenolepis fraterna* (Stiles), to free them from adherent bacteria and keep them physiologically active in artificial media. Surface sterilization was attained by five minutes exposure to a 10% saline solution of silver protein (Merck), or by the sedimentation method of washing in saline. No success was obtained with bacteriological media but in a dilution of Baker's tissue culture medium A (10 drops to 5 cc. of Tyrode's solution), *Hymenolepis fraterna* remained active for 20 days, considerably exceeding its normal longevity *in vivo*.

1. Introduction

The technique of keeping intestinal worms alive and physiologically active when outside their normal hosts is rudimentary, and with tapeworms the results obtained have been disappointing owing to their extreme fragility, their extreme restriction of habitat, and their susceptibility to bacterial attack. The problem is made particularly difficult by the absence of information concerning their alimentary and respiratory processes.

The problem is to establish a non-lethal method of freeing the tapeworm surface from adherent bacteria, after removal from the host, and to establish a nutrient medium in which the tapeworm will remain physiologically active for a period at least as long as the normal life period spent within the host.

For a summary of the results so far obtained, reference may be made to Wardle (8, 9, 10, 11). It may be said, briefly, that greater success has been obtained with larval tapeworms than with adults, but in no case has true growth been observed, and the success of such experiments has to be estimated by the length of time that the worms remain actively undulant. The maximum values so recorded fall considerably short of the length of life within the host environment where this latter value is known.

2. Tapeworm Sterilization

Two methods of sterilizing the tapeworm surface are available: (1) exposure to saline dilutions of bactericidal substances; (2) washing with sterile saline solutions, either by simple rinsing or by *sedimentation*, that is to say, by allowing the worm to fall repeatedly through columns of sterile saline so that the lighter bacteria are left suspended in the upper layers of the solution. Few observations have been recorded as to the effect of bactericides upon tapeworm viability. Coutelen (4) has noted the lethal effect of mercuric chloride (1 : 1000) and formalin (1 : 200) upon larval *Echinococcus*; Clapham (1),

¹ Manuscript received April 24, 1941.

Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada.

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the lethality of hexyl resorcinol in dilutions of 1 : 1000 to 1 : 20,000 upon *Hymenolepis fraterna* (Stiles); Wardle (9), the adverse effect of a wide range of common bactericides upon the longevity of larval *Nybelinia surmenicola* Okada.

In the present experiments tests were made with a number of organometallic bactericides recommended for sterilizing mucous membranes; in addition, tests were made with resorcinol, phenol, trikresol, and chloramine T. These substances were dissolved in physiological saline (Tyrode's solution) in percentage proportions of 0.0001, 0.001, 0.01, 0.1, and 1.0. They were tested upon the common sheep tapeworm, *Moniezia expansa* (Rud.). The scolex and neck region were snipped from each adult worm (as soon as possible after removal from the sheep), rinsed with warm sterile saline solution, then transferred (to test the effect on the worm) to sterile Tyrode's solution, or transferred (to test the degree of bacterial inhibition) to agar plates. The worms were subsequently kept at 37.5° C.

Untreated worms, kept in Tyrode's solution, did not remain actively undulant for more than 24 hr. On agar plates they became septic within 48 hr. The maximum periods of undulant activity among the treated worms are given in Table I. At all higher concentrations of the above substances,

TABLE I

MAXIMUM PERIODS OF UNDULANT ACTIVITY OF WORMS TREATED BY VARIOUS STERILIZING SOLUTIONS AND CULTURED IN VARIOUS NUTRIENT MEDIA

Sterilizing solution	Concentration, %	Period of activity, hr.
Silver protein	0.1	168
Chloramine T	0.1	144
Argyrol (A. C. Barnes)	1.0	96
Chinosol (Burrows Wellcome)	0.01	96
Mercurrescin (Ingram and Bell)	0.1	96
Phloroglucin	0.1	72
Resorcinol	0.01	48
Trikresol (Schering)	0.01	48
Phenol	0.01	48
Neosilvol (Parke Davis)	0.1	24
Nutrient media		
Tyrode's solution		168
Nutrient agar and hog serum		120
Hog serum		48
Nutrient agar (saline)		48
Heart infusion broth (aqueous)		48
Nutrient gelatine (aqueous)		33
Heart infusion broth (saline)		24
Gastric mucin		12
Dextrose broth (aqueous)		9
Tryptose phosphate		Lethal (immediately)
Kracke's blood culture medium		Lethal (immediately)

and in *all* concentrations of merthiolate (Eli Lilly and Co.), the worms died immediately. After exposure to the relatively non-lethal dilutions, complete bacterial freedom seemed to have been conferred (to judge by the subsequent absence of bacterial contamination of the agar plates) by silver protein, mercurcescin, chloramine *T*, argyrol, and phloroglucin. Bacterial contamination, after 24 hr., appeared in plates containing those worms that had been exposed to neosilvol, resorcinol, phenol, and trikresol.

The bacterifugal effect of simple rinsing with sterile saline solution was tested upon larval *Diphyllobothrium latum* (L.), separated from adherent fish muscle by soaking overnight in Tyrode's solution at 37.5° C. After rinsing with sterile saline, the worms were removed in batches of 10 to common bacteriological media (see Section 3). No subsequent bacterial contamination of the media was observed within 48 hr., but as the maximum periods of undulant activity did not, with one exception, exceed 48 hr., the experiments must be regarded as inconclusive. In Tyrode's solution alone, worms rinsed in such a manner lived 168 hr. without perceptible sepsis.

The sedimentation method of obtaining freedom from bacteria—used with success by Cleveland (3) for protozoa, Ferguson (5) for trematode cercariae, and Glaser and Stoll (6) for nematode worms—was tested upon specimens of *Hymenolepis nana* var. *fraterna* (Stiles). This is a small worm common in wild rats and peculiar in being able to carry out its whole life cycle within the host; rats can be infected by being fed eggs or whole tapeworms, and, despite the short life period within the host, a strain of tapeworms can be maintained indefinitely in laboratory animals. The small size of this worm (less than 50 mm.), its availability in rats throughout the year, and its marked undulant activity, make it a most suitable subject for experiments of this nature. After removal from the host, the hymenolepids were washed by being allowed to fall 10 times in succession through columns of sterile Tyrode's solution. Four-inch test tubes were used, the supernatant fluid being poured off before the worms were removed from the bottom of the tube and transferred to a fresh tube. Final transference was made to sterile media in small Petri dishes. In sterile Tyrode's solution, the worms, after sedimentation, remained active up to nine days. In Baker's Medium *A*, some remained sterile and active up to seven days, but some became septic within 24 hr. In the very dilute medium finally adopted (see Section 3), they remained sterile up to 20 days, the maximum period of undulant activity recorded.

3. Nutrient Media

In the absence of information as to their food requirements, attempts to feed tapeworms *in vitro* cannot be other than empirical. Wardle (9) describing experimental cultivation of larval tapeworms on a range of saline and semi-

solid media, suggests that from the standpoint of retention of normal appearance and of movement, rather than duration of undulant activity, nutrient gels under aseptic conditions offer the best possibilities for tapeworm cultivation.

In the present experiments the nutrient media tested were liquid and comprised a series of Difco bacteriological media and the tissue culture medium known as Baker A (2).

In one series of experiments, plerocercoid larvae of *Diphyllbothrium latum*, after being rinsed with sterile saline, were transferred to a range of nutrient broths made up according to the manufacturers' directions, in water or saline, and sterilized by Seitz filtration. The cultures were incubated at 37.5° C. The maximum periods of undulant activity recorded are shown in Table I. One specimen in nutrient agar plus hog serum grew to a length of 50 mm., which is three times the maximum length recorded among 100 measured plerocercoids.

In a second series of experiments, hymenolepid worms, after sedimentation, were transferred to dilutions of the tissue culture medium, Baker A, which is essentially a solution of Witte's peptone (0.675%) in a balanced saline solution containing small quantities of cysteine hydrochloride, hemin, insulin, thyroxine, glucose, vitamin A, ascorbic acid, and glutathione; immediately before use, 10% of ox serum was added.

In Tyrode's solution alone the worms remained active up to nine days; in Baker's medium alone, they remained active up to seven days, but the medium showed bacterial clouding and had to be renewed every two days. In mixtures of Baker's and Tyrode's in proportions 1 : 3, 2 : 2, 3 : 1 they remained active up to three days. The most satisfactory combination was found to be a very dilute solution of Baker's medium in Tyrode's solution, 10 drops of Baker's to 5 cc. of Tyrode's. In this medium, undulant activity without bacterial clouding was maintained for as long as 20 days.

4. Conclusions

Although the average length of life of *Hymenolepis fraterna* in the rat is not known with certainty, there is a general belief among students of the question that the postmaturation period of the life cycle is brief. Shorb (7) gives an average value of 11 to 16 days for the prematuration phase of the life cycle, 11 days for the postmaturation phase.

Maintenance of mature *Hymenolepis fraterna* in an artificial medium for as long as 20 days must be regarded therefore as a satisfactory test of the suitability of the medium selected and of the preliminary treatment for freeing the worm from adherent bacteria.

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THE RATE OF GROWTH OF THE TAPEWORM *DIPHYLLOBOOTHRIUM LATUM* (L.)¹

BY ROBERT ARNOLD WARDLE² AND NANCY KATHARINE GREEN³

Abstract

Observations on scolex length, total weight, total length, and weight-length ratio, of specimens of *Diphyllbothrium latum* (L.), reared in dogs, suggest that the increase in scolex length as the worm ages is due to deterioration of the scolex musculature; that increases in weight and length during the first six days within the host are due to water absorption; that from the sixth day to the 15th day, growth is rapid and may be expressed by the formulae:—

$$W \text{ (weight)} = (0.18)e^{0.61n}, L \text{ (length)} = (2.3)e^{0.39n}, \\ \text{and } W/L \text{ (weight-length ratio)} = (0.08)e^{0.22n}$$

where e is the natural logarithm base and n is the age in days. After the 15th day, the values of W/L fluctuate rhythmically, the high values corresponding to periods of strobila apolysis, the low values corresponding to periods of egg discharge. Examination of a wide range of specimens from man and dog suggests that, with exceptions, the average weight-length ratio fluctuates between values of 3.0 and 5.0 in mature egg producing specimens.

1. Introduction

Information concerning the rate of growth of tapeworms is scanty. They cannot be continuously observed whilst within their hosts. They cannot be kept alive for more than a few days outside such hosts. The rate of growth must, therefore, be inferred by measuring groups of individuals of known ages, and the study of tapeworm growth is limited to such tapeworm species as can be reared in laboratory animals or in man. The only published observations on tapeworm growth are those of Petruschewsky and Tarassow (3, 4) on *Diphyllbothrium latum* (L.), of Penfold, Penfold, and Phillips (2) on *Taenia saginata* Goeze, and of Chandler (1) on *Hymenolepis diminuta* (Rud.).

The choice of a criterion of growth changes in tapeworms is restricted by the absence, in these animals, of exoskeleton, endoskeleton, and osmotic rigidity. The living worm rhythmically contracts and expands; it is freely permeable to water so that weight and length are affected by the nature of the medium in which it is examined or preserved; commonly, only a fragment of a tapeworm may be available for examination.

Linear length, used as a criterion of growth by previous authors, whilst applicable to small tapeworms such as hymenolepid species or plerocercoid larvae, is less applicable to large tapeworms and not applicable at all to fragments; a similar objection may be raised against the value of *total weight*.

The value of *average cross sectional area*, calculated from average length, average weight, and average specific gravity, offers the advantage of applic-

¹ Manuscript received April 24, 1941.

Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Man., with financial assistance from the National Research Council of Canada.

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ability to fragments. In the case of *Diphyllbothrium latum*, preserved in 5% aqueous formalin, the specific gravity fluctuates narrowly on either side of unity and the average cross sectional area may be regarded as equivalent to the weight-length ratio.

2. Methods

Specimens of *Diphyllbothrium latum*, obtained from fish, were reared in young dogs of similar breed, age, weight, size, and genetic origin. Each dog was infected with five plerocercoids of *Diphyllbothrium latum*, all dogs being infected at the one time with plerocercoids taken from one batch of fish. The dogs were killed at intervals of 3 to 30 days after initial infection and the worms were removed, killed in hot 5% formalin, preserved and afterwards measured in the same medium. From 60 plerocercoids, 39 worms were thus recovered. Measurements were made of the scolex length and of the total length of worms ranging from 3 to 75 days and 3 to 98 days of age, respectively.

Tapeworms killed and preserved in a hypotonic medium absorb water readily and the values of length and weight observed can be compared only with those observed for other worms killed and preserved in the same way. Formalin fixation offers advantages over ice water immobilization of the living worm in permitting comparison with casually collected specimens which almost invariably are fixed and preserved in weak formalin; it also permits lengthy storage of specimens with little change beyond a negligible alteration in weight due to oxidation of part of the phospholipid content.

Little advantage is gained in superinfecting dogs with large numbers of plerocercoids in the hope of gaining large numbers of adult worms. Such superinfected hosts tend to lose the majority of their parasites. Among dogs superinfected with 30 to 50 plerocercoids, the majority lost their worms completely after the 25th day; the greatest number recovered from any one dog was 27, after 75 days' infection. One dog initially infected with 30 plerocercoids had only eight worms at the end of 98 days. It may be noted, that a low number of tapeworms per individual has also been recorded by Petruschewsky and Tarassow (3) in a northern Russian population exposed to superinfection with *Diphyllbothrium latum*. Moreover, the length values of worms from superinfected dogs contrast unfavourably with those of worms from lightly infected animals. Chandler (1) found that the average length of *Hymenolepis diminuta* in laboratory rats is in inverse proportion to the number of worms present, ranging from an average of 1000 mm. in single-worm infections to 300 mm. in 40-worm infections.

3. Discussion of Data

In Table I are given average scolex lengths of larval specimens of known ages, killed in hot 5% formalin. The data suggest a progressive increase in length as the worm grows older. Among plerocercoids whose scolices have been everted by immersing the worms in physiological saline, the scolices are

TABLE I
AVERAGE SCOLEX LENGTHS OF *Diphyllobothrium latum* (L.) OF KNOWN AGES

Age, days	Number	Range of lengths, mm.	Average length, mm.
3	7	0.368 - 0.828	0.526
6	5	0.736 - 0.828	0.797
9	5	0.644 - 0.736	0.690
12	3	0.736 - 0.920	0.797
15	3	0.736 - 0.828	0.770
18	2	0.828 - 1.104	0.966
21	3	0.736 - 1.104	0.889
24	7	0.736 - 1.288	0.999
27	2	0.920 - 1.192	1.104
30	3	0.937 - 1.945	1.104
56	24	1.020 - 2.400	1.523
75	29	1.125 - 2.400	1.700

extremely mobile, expanding to a length of 1.5 to 2.0 mm. by muscular contraction, then contracting through muscular relaxation to a length of only half that value. When killed in hot formalin, the scolex remains digitate or lanceolate in shape and at its minimum value of length. As the worm grows older, such mobility is less marked, owing apparently to a degeneration of the scolex musculature. The scolex when expanded is never so long, when contracted never so short, as is the newly everted one. In an old worm, the range between expanded and contracted length diminishes until the scolex seems almost immobile, and living or dead, remains club-shaped, spoon-shaped, or almond-shaped. The effect is thus one of increasing scolex length as the worm grows older.

The function of the scolex is to anchor the early strobila to the host gut wall. In the older worm, maintenance of position in the host gut is effected by muscular tonus, by pressure of the powerfully muscular strobila against the gut wall; the scolex serves little purpose, adheres only loosely to the gut wall, and any increase in its size and development is not to be expected.

Table II presents the data obtained for weight and length and weight-length ratio of entire worms, in each age group. Between the maximum and minimum values of weight and length, there is in each age group a relatively wide spread, and it does not seem possible to determine the possible age of a fragment of *Diphyllobothrium latum* by inspection of the weight-length ratio. Such variation in weight and length within each age group arises in part from the corresponding variation of the plerocercoids used for initial infection.

The lengths of 56 plerocercoids, killed, preserved, and measured in 5% formalin, ranged from 1.0 to 17.5 mm.; 41 of these had lengths between 1.5 and 6.5 mm., the average length being 3.7 mm. Fifty plerocercoids ranged in weight between 0.1 and 7.16 mg.; 36 of them had weights between 0.3 and 2.0 mg., the average weight being 1.4 mg. The *W/L* value of 0.4 is thus much higher for the plerocercoid than for the worms between one and

TABLE II

AVERAGE WEIGHT, LENGTH, AND WEIGHT/LENGTH VALUES FOR *Diphyllbothrium latum* (L.) REARED IN DOGS, AND KILLED AND PRESERVED IN 5% FORMALIN

Age, days	Number	Average weight, mg.	Average length, mm.	Weight/length ratio
5 plerocercoid infections				
3	7	1.44	7.09	0.203
6	5	5.30	20.20	0.262
9	5	43.20	81.20	0.532
12	3	276.30	250.25	1.103
15	4	2350.00	659.00	3.57
18	3	3890.00	698.00	5.57
21	4	2660.00	664.00	4.01
24	3	1676.00	266.20	6.30
27	2	2215.00	660.00	3.36
30	3	9215.00	1436.00	6.42
30 to 50 plerocercoid infections				
32	4		256.00	
41	4		1250.00	
50	1		765.00	
56	24		385.00	
65	10		360.00	
75	27	1850.00	558.00	3.32
98	8		1688.00	

six days old. If, however, the relatively high specific gravity, of 1.38, of the plerocercoid stage be taken into consideration, W/L becomes 0.274 which compares closely with that of the one-day to six-day worms. It is possible, therefore, that no true growth of the young worm, in the sense of new tissue formation, occurs within the first six days, but that the apparent increase in weight and length is due to water absorption. It may be noted that Chandler (1) found very slow linear growth in *Hymenolepis diminuta* during the five-day to seven-day period in the host, as compared with the rapid growth during the 7- to 18-day period. The average length value of 20 mm. (given in Table II) for the six-day old worms may be contrasted with the value of 27.0 mm. found by Petruschewsky and Tarassow (4) for *Diphyllbothrium latum* (L.) of the same age in a dog.

Without further knowledge of the food requirements of *Diphyllbothrium latum* conclusions cannot be drawn as to the influence of its position in the host gut upon available food supply and upon its consequent weight and length. Among 80 worms, ranging from 3 to 98 days old, only 5% lay in the first quarter of the host gut, measured between pylorus and ileocolic valve; 37.5% lay in the second quarter; 22.5% in the third quarter; the remaining 35% in the fourth quarter. In the case of *Hymenolepis diminuta*, Chandler (1) found that the worms take up their initial position far back in

the gut and subsequently move forward. Our observations upon the position of young specimens of *Diphyllobothrium latum*, in kittens, showed that after 13 hr. from initial infection the worms lay at distances of 7, 11, 12, 13, 16 in., respectively, from the pylorus in a gut measuring 33 in. between pylorus and ileocolic valve; after 25 hr., 17 worms lay at distances between 7.5 and 36 in. from the pylorus in a gut measuring 56 in. between pylorus and ileocolic valve. These observations suggest that scattering of the individuals of an age group along the gut is as characteristic of the first few hours of infection as of the later days of infection.

The data of Table II suggest that during the period 6 to 15 days after initial infection, growth is rapid and progresses geometrically according to the general formula $G = (A)e^{kn}$ where G is the growth value, A and k are constants, e is the natural logarithm base, and n is the age in days. The

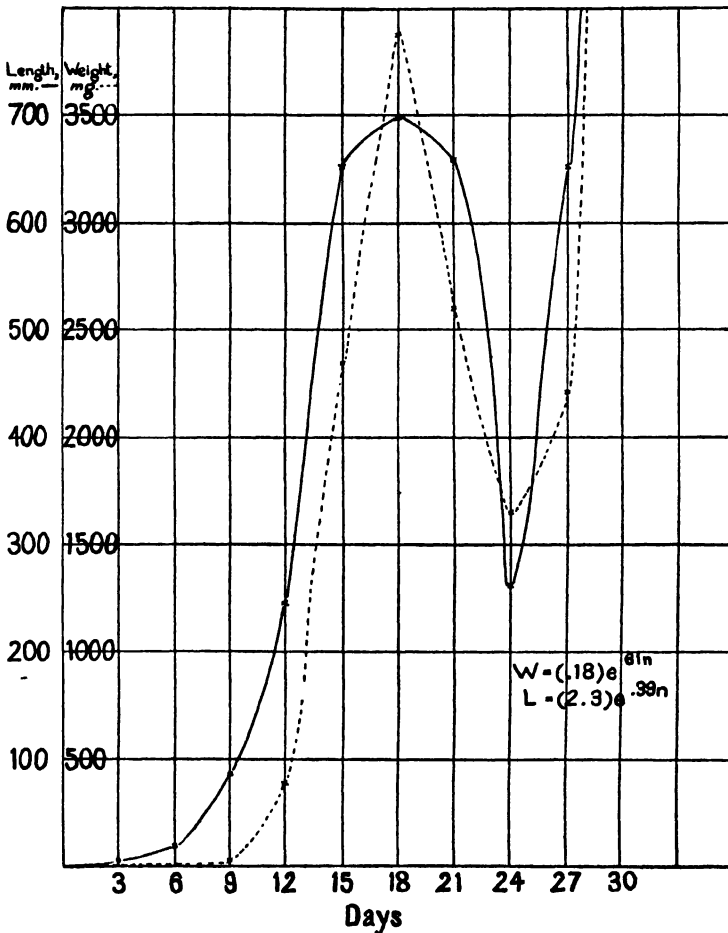


FIG. 1. Increase in length and weight of specimens of *Diphyllobothrium latum* (L.) in dogs

actual values of weight (W), length (L), and weight-length ratio (W/L) recorded fit the formulae:—

$$W = (0.18)e^{0.61n}, L = (2.3)e^{0.39n}, \text{ and } W/L = (0.08)e^{0.22n}.$$

During this 6- to 15-day period the average daily increment of length was 48 mm. During the 15- to 30-day period it was 61.5 mm. Petruschewsky and Tarassow (4) for the 6- to 36-day period, found the average daily increment of length to be 47 mm. for *Diphyllbothrium latum* in a dog, 150 mm. for a specimen in a man.

As may be seen in Fig. 1, the value of W/L reaches a peak on the 18th day after initial infection. This peak coincides with the appearance in the posterior proglottids of the first batch of eggs. In *Diphyllbothrium latum*, whether in man or dog, the first discharge of eggs occurs between the 18th and 20th day; the exhausted proglottids diminish to one-half or one-third of their former weight, eventually disintegrate, and break free from the strobila.

In Table II this first egg discharge is clearly indicated by the fall in W/L value in the 21-day specimens. The first apolysis, or shedding of exhausted tissue, is indicated by the rise in W/L value in the 24-day specimens. Subsequent egg discharges are indicated by lower W/L values; subsequent apolyses are indicated by heightened W/L values.

The conclusion may be drawn that egg discharge and tissue apolysis are not continuous phenomena, but occur, as it were, spasmodically at intervals which, up to 30 days after initial infection, appear to be of a duration of three days. The W/L values thus rise and fall between certain limits. An examination of a large number of fragments of *Diphyllbothrium latum* from man and dog, from various localities, suggests that these limits are approximately 3.0 and 5.0. Outstanding exceptions were provided by some specimens from man. One enormous specimen from Leningrad had a W/L value of 11.0 and two from Saskatchewan had values of 7.55 and 7.83, respectively. A Russian specimen, known to be 36 days old, had a W/L value of 5.70. Clearly any metabolic features of the host that stimulate egg production in *Diphyllbothrium* will produce an unusually high W/L value; those that suppress egg production whilst stimulating linear growth, will lead to an unusually low W/L value, but the nature of the metabolic features is at present unknown.

The 30-day period of the experiment was too short to indicate whether new growth exceeds or merely keeps pace with apolytic loss. Penfold, Penfold, and Phillips (2), studying *Taenia saginata*, found an increase of 9 to 12 proglottids per day, and a loss of 8 to 9 proglottids per day, growth thus exceeding apolysis.

In an apolytic tapeworm, growth during early life may be expected to exceed apolytic loss, whereas later in life it should merely equal it and later still should lag behind it.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

SEPTEMBER, 1941

NUMBER 9

A PHOTOELECTRIC FLUORIMETER¹

By A. H. WOODCOCK²

Abstract

A fluorimeter designed for the determination of vitamin B₁ as thiochrome and riboflavin is described. The instrument is entirely self-contained and simple to operate. It is capable of detecting thiochrome in concentrations as low as 0.005 microgram per ml. and sufficiently stable to permit measurement of higher concentrations. By using vacuum type photocells an electric eye can be used as a null indicator and the comparatively expensive galvanometer is eliminated.

Introduction

In recent times much stress has been laid on the importance of two members of the vitamin B complex, thiamin and riboflavin, in relation to nutrition. It has been found that an oxidation product of thiamin, thiochrome, and riboflavin fluoresces when exposed to light of suitable wave length. This fluorescence forms the basis of determining these substances by chemical methods (3). Although instruments for making fluorescent measurements are carried by most manufacturers of laboratory apparatus, and others have been already described (1, 2, 4), it is felt that the one described in this paper fills a need for an inexpensive instrument with good sensitivity.

Description of Instrument

The instrument is entirely self-contained in a brass case measuring 12 × 12 × 7 in. A plan and elevation drawing are shown in Fig. 1. The source of light is a commercial high pressure mercury arc lamp from which the light is separated into two beams at right angles. The first beam passes through a filter that eliminates all but the ultra-violet light which is used to activate the sample of fluorescent solution. A second filter that eliminates ultra-violet light is employed behind the solution so that only the fluorescent light causes a photoelectric current in the cell behind it.

The second beam of light passes through an initial regulating diaphragm and a filter that, like the first, transmits only ultra-violet light. The light then enters a system of polarizing and analysing Nicol prisms, and finally a second photocell. The analyser, determining the amount of light entering the photocell, is adjusted through a worm gear and its angular position is measured on an illuminated scale.

¹ Manuscript received May 29, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada. Published as N.R.C. No. 1009.

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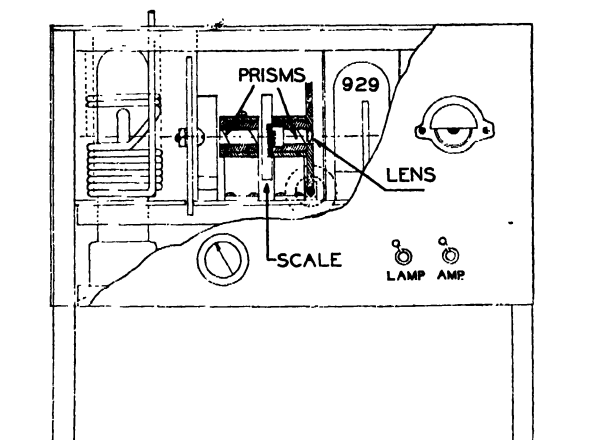
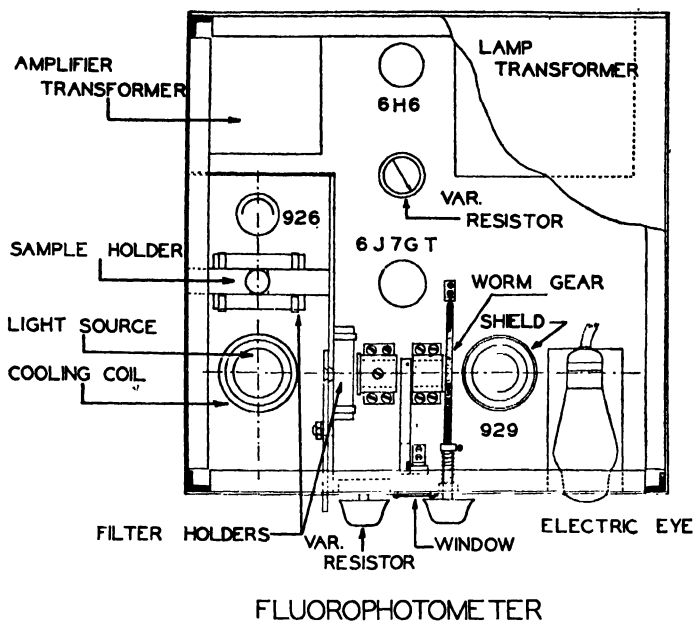
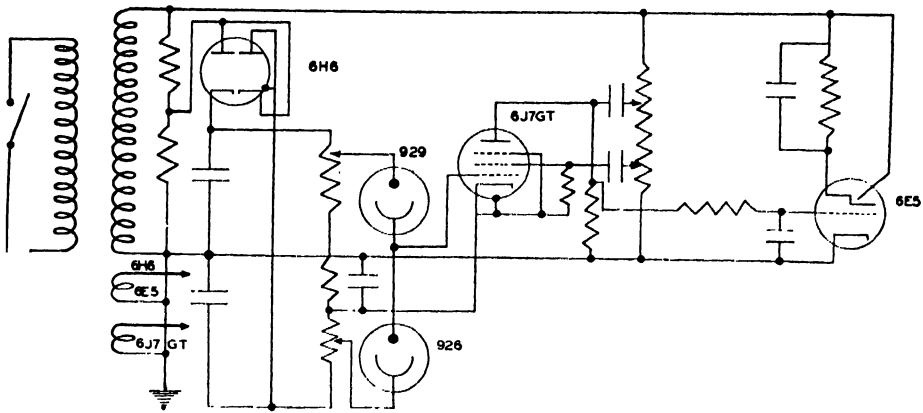
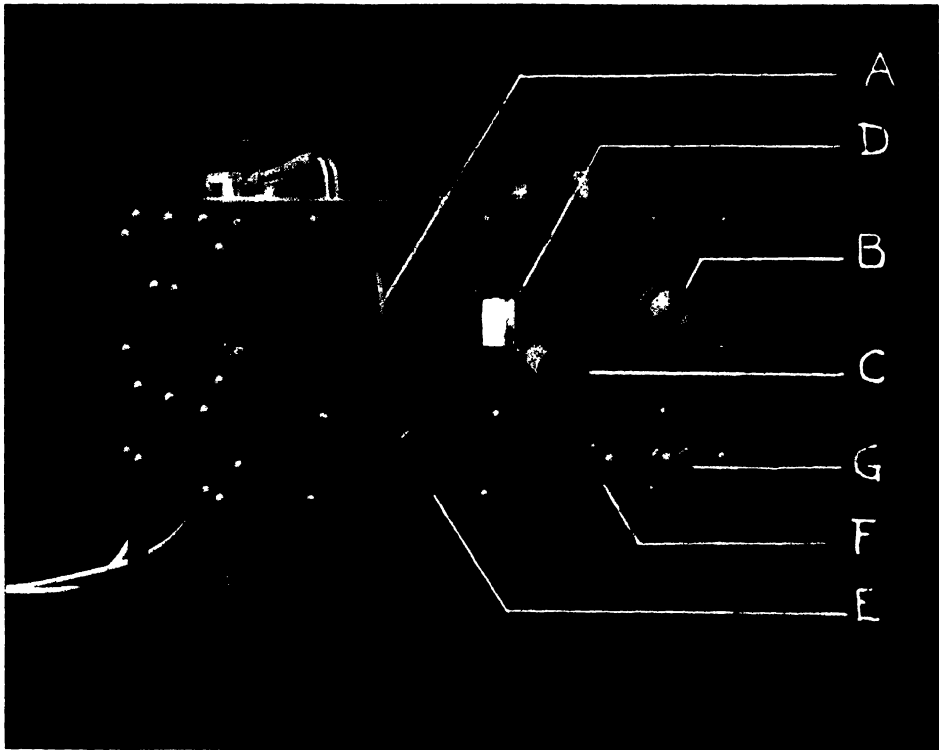


FIG. 1. Plan and elevation drawings.

The electrical circuit is shown in Fig. 2. The two photoelectric cells are connected to the amplifier in opposition so that any difference in photoelectric currents is registered on an electric eye. Any variation in the brightness of the source does not affect the balance since the photoelectric current varies equally in both cells. The sensitivity of this circuit is limited by the insulation leakage of the photoelectric cells, which is very small. However, this leakage is used to obtain an initial balance with sample absent and Nicol prisms crossed by regulating potentiometers supplying the voltage to the cells. In practice only one of these is used, the other being set at a predetermined value.

FIG. 2. *Electrical circuit.*

A photograph of the instrument is shown in Fig. 3. *A* and *B* are the regulating diaphragm and the electric eye, respectively. Knob *C* is used to adjust the analysing Nicol prism, the rotation of which is read on scale *D*. Knob *E* is the zero adjustment of the electric eye, while *F* and *G* are switches for the light source and amplifier, respectively.

FIG. 3. *Photograph of instrument.*

Theory and Performance

The intensity of light transmitted by Nicol prisms is given by

$$I = I_0 \sin^2 \theta$$

where θ is the angle of rotation of the analyser from the crossed position or zero intensity, and I_0 is the maximum intensity. The amount of fluorescent light produced by the sample is therefore proportional to $\sin^2 \theta$ and would be expected a priori to be proportional to the concentration of fluorescent substance present. To test this, calibration curves have been plotted on logarithmic scales between concentration and $\sin^2 \theta$ for thiochrome and riboflavin dissolved in 20% ethyl alcohol; these are shown in Figs. 4 and 5, respectively. The departures from linearity at extreme dilutions are probably due to fluorescent impurities in the solutions whereas the decrease in $\sin^2 \theta$

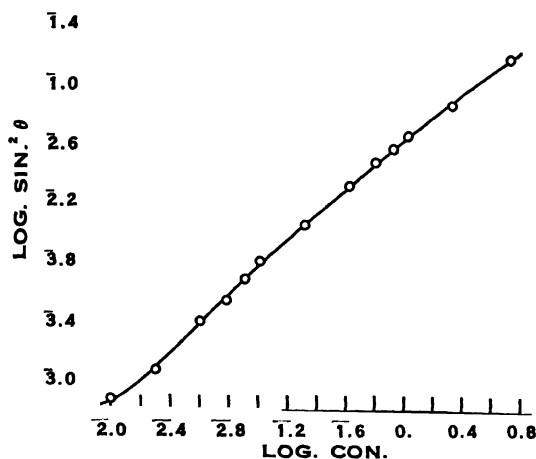


FIG. 4. Calibration curve for thiochrome plotted on logarithmic scale.

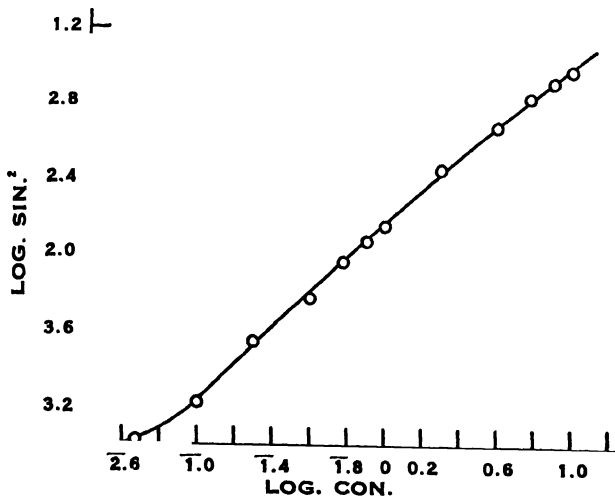


FIG. 5. Calibration curve for riboflavin plotted on logarithmic scale.

below the theoretical values at higher concentrations can be explained by absorption of ultra-violet light by the sample. At very much higher concentrations visual inspection confirms this and shows that the fluorescence is confined to the side of the test tube at which light enters.

It will be seen that both the sensitivity and accuracy are satisfactory for ordinary work. The instrument has also proved to be quite stable and determinations made on the same solution from day to day agree with each other within the limits of error of the instrument.

The desire to keep the cost of the instrument low has been considered throughout the design, the total price of equipment and material being less than \$100.00. The main factor in reducing this figure to a low value is the use of the vacuum type of photoelectric cell which permits the use of an amplifier and the elimination of a delicate galvanometer. Thus a rugged, stable instrument has been produced which is extremely simple and rapid to operate.

Acknowledgment

The author wishes to acknowledge the skilled assistance of Mr. H. Tessier who constructed the instrument.

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THE BREEDING CYCLE IN *SAGITTA ELEGANS ARCTICA* AURIVILLIUS¹

BY M. J. DUNBAR²

Abstract

From size distributions of populations of *Sagitta elegans* in waters of the Canadian eastern Arctic, and from examination of the gonads, it is shown that *Sagitta* has a breeding cycle of an alternating, or two-phase type, such that two generations, separated in age by one year, exist at the same time.

This breeding cycle is contrasted with the known habits of *Sagitta* in temperate waters; in the English Channel there are several broods produced per year, and along the Atlantic coast of North America there is one long breeding period in the summer, with apparently only one brood involved. There is also a contrast between the size distributions described here and those found by the author in Disko Bay, west Greenland; this is tentatively correlated with hydrographic differences between the two regions.

The two-phase cycle is evidently effective in ensuring the occurrence of large numbers of the species under conditions of slow growth. Rigid maintenance of the cycle, without the development of a lag or distortion, would result in a genetic isolation of the two broods that might lead to differentiation and possibly to speciation.

Introduction

The great abundance of phytoplankton in polar waters during the summer must be considered an important factor in the development of the great quantity of zooplankton found in these regions, but the cold water which, by its ability to contain large quantities of dissolved gases and its encouragement of vertical movement in the early spring, plays an important part in the growth of the phytoplankton, has also the effect of slowing down growth rates. To explain the abundance of planktonic life, therefore, some mechanism, calculated to overcome the check on population consequent upon this slow growth, should be demonstrable. Loeb (10), working on sea-urchin eggs and young embryos, found that the temperature coefficients of the length of life and of the speed of development to maturity were very different; for the range of 32° to 20° C. a drop of 1° C. approximately doubled the length of life, whereas the rate of development was only slightly altered. He offered this result as an explanation of the quantity of plankton in polar seas, by pointing to the clear inference that many generations must exist at the same time.

Most planktonic organisms, however, seem to die after spawning, which upsets Loeb's calculations. Lindsey (9) has made the point that the Loeb effect cannot be true of the phytoplankton, since the parental protoplasm is used up in the formation of the next generation. Some other mechanism must therefore be sought. The study described below offers such a mechanism for a member of the zooplankton, by showing that in *Sagitta*

¹ Manuscript received May 2, 1941.

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elegans arctica the check on population resulting from the slow growth rates in the very cold water is offset by a two-phase breeding cycle, so that, as Loeb suggested, two generations, and during the breeding period three generations, do exist at the same time, though their coexistence is evidently not due to the Loeb effect.

Material

Sagitta elegans Verrill has received much attention in recent years from workers on both sides of the Atlantic. It is a convenient indicator of water movements, and is simple to handle, dissect, and section. The form *arctica* Aurivillius is larger than the typical *elegans* form, has a shorter tail segment, and is found in Arctic seas, being particularly abundant near the coasts. It has also been recorded by Fraser (5) from the Firth of Forth, in Scotland. The present material was collected in the summers of 1939 and 1940, in the waters of the Baffin Island coast and northern Labrador.

The positions of the stations mentioned in this work are as follows:

Clyde River: 70° 20' N., 69° W. East Baffin Island.

Pangnirtung: 66° N., 66° W. East Baffin Island.

Frobisher Bay: 63° 20' N., 76° 30' W. Southeast Baffin Island.

(Also a station 60 miles S.E. of this position.)

Gabriel Strait: 62° N., 65° 30' W. Southeast Baffin Island.

Lake Harbour: 62° 40' N., 69° 40' W. South Baffin Island.

Hebron: 58° 20' N., 62° 30' W. Northern Labrador.

The water at all these stations is polar; there is no evidence for the presence of any Atlantic water at all. The plankton is typically arctic, including such indicator forms as *Oikopleura vanhoeffeni* Lohmann, *Calanus hyperboreus* Kröyer, *Diphyes arctica* Chun, *Mertensia ovum* Fabricius, *Themisto libellula* Mandt, *Limacina helicina* Phipps, and others. Temperatures and water samples were taken at four of the above stations, namely, Clyde River, Pangnirtung, Frobisher Bay, and Lake Harbour. The salinities ranged from 32.93 o/oo to 28.04 o/oo, and the temperatures, from 10 metres down, between 1.0° and -1.39° C., with the surface temperature as high as 3.68° C. on one occasion.

A one-metre stramin net was used at all stations, with a small-mesh silk net for diatoms, which occasionally picked up specimens of *Sagitta*. During the seasons of collecting, about 4000 specimens were taken, the bulk coming from the six stations listed here. All the specimens from these stations have been measured, with the exception of a few damaged individuals, and with the exception also of some taken at Pangnirtung in 1939. Here one 10-minute haul produced 2613 *Sagitta*; a sample of 958 specimens was taken for measurement. The tail fin was not included in the measurements.

Horizontal tow-nettings were made at various depths, from the surface to 35 metres. Vertical and oblique hauls reached to about 75 metres. The bulk of the *Sagitta* population in the fjord waters was found in the upper 35 metres, in the daytime below 15 metres.

Size Distributions and Breeding Cycles

The size frequencies are shown in Figs. 1 to 7, and in Table I. The movements of the *S.S. Nascope* made it impossible to work the whole season at one place, which would have been the best procedure. Hence the material from six stations has been collated, contrasting the condition in August at one station with that in September at another. In Fig. 2, for instance, material from two stations, Hebron and Pangnirtung, is plotted on the same graph, the catches from the two stations representing two separate size groups. Fig. 6 is made up from Figs. 3, 4, and 5. Thus the whole region of investigation, being of one type of water, has been treated as a unit, the assumption being that at any one time the *Sagitta* population over the whole area will be at approximately the same stage in the cycle.

It will be seen that none of the distributions is normal, and that more than one mode is apparent in all of them. This is taken to mean that more than one brood is present in the population. To analyse the curves statistically, to prove what is apparent at sight, is unnecessary and unsatisfactory.

It may be taken that *Sagitta elegans arctica* has only one breeding period in the year; this conclusion is drawn not only from the present results, but from other work in this field by Kramp (7 and 8), Ussing (17), Huntsman and Reid (6), and Dunbar (3). The size groups have therefore been classified (Figs. 1, 2, 6, and 7) as follows:

Group A. The group breeding in the year in which they were caught.

Group B. The adolescent group, due to spawn in the following year.

Group C. The young group, the offspring of Group *A*, due to spawn two seasons later.

The state of the gonads of Groups *A* and *B* supports this interpretation. The condition of the ovaries can be seen by external examination, using transmitted light, and the approximate state of the testes can be gauged from their transparency, and by staining. To obtain a more exact knowledge of the testes, however, 12 individuals from Frobisher Bay, Pangnirtung, and Clyde River were sectioned and stained with Harris's haematoxylin. The gonads of Group *B*, in 1940, (Figs. 6 and 7) were all unripe; the ovaries were very small, with small cells, or so rudimentary as scarcely to be visible. The tail cavities were clear, and on being sectioned showed only small testes closely apposed to the lateral walls of the cavities. At the beginning of spermatogenesis in *Sagitta*, groups of sperm mother cells, the "polyblasts" of Bordás (1), are budded off from the testes and circulate in the tail cavities (Burfield (2)). There were no polyblasts present in the *B* groups from Frobisher Bay and Clyde River. The seminal vesicles, moreover, were not developed.

The same was true of the gonads of the 1939 *B* group from Pangnirtung (Fig. 2), in those animals under 30 to 31 mm. in length. Those above this limit showed polyblasts in the cavities, and had larger ovaries. From a comparison with Fig. 7 it seems likely that some individuals of Group *A*

TABLE I
SIZE DISTRIBUTION IN *Sagitta elegans arctica* AURIVILLIUS

Pivotal length (mm.)	Frequency						
	1939			1940			
	Lake Harbour*	Pangnirtung†	Hebron†	Gabriel Strait*	Frobisher Bay*	Lake Harbour*	Clyde River†
4							2
5	2		4				2
6	2		34				1
7	8		52				4
8	2		51				28
9	2		63	1	1		36
10			37			3	45
11			14			1	40
12			6			1	29
13			2				14
14			2			1	2
15				1		2	1
16		1				2	4
17		2			1		18
18		6			1	3	22
19	1	12		1		6	27
20		21		2	2	3	32
21	2	33		1	3	10	25
22	1	60		1	2	8	18
23	4	80		1	2	12	19
24	1	149		4	2	11	12
25	5	123		7	3	13	8
26	5	127		5	7	7	7
27	6	100		3	9	5	6
28	7	70		3	8	4	4
29	4	62		1	5	4	1
30	15	26		5	7	5	5
31	11	17		1	13	7	7
32	9	16		2	9	7	8
33	9	13		3	21	16	7
34	13	13		3	30	16	4
35	8	5		3	29	6	
36	10	4		5	32	9	3
37	5	7		5	25	10	
38	3	5		2	20	6	
39		3		1	17	8	
40	2	1		2	8	1	
41	1	1			1		
42		1			2		
43	1			2	1		
44					2		
45	1						
51	1						

* July-August.

† September.

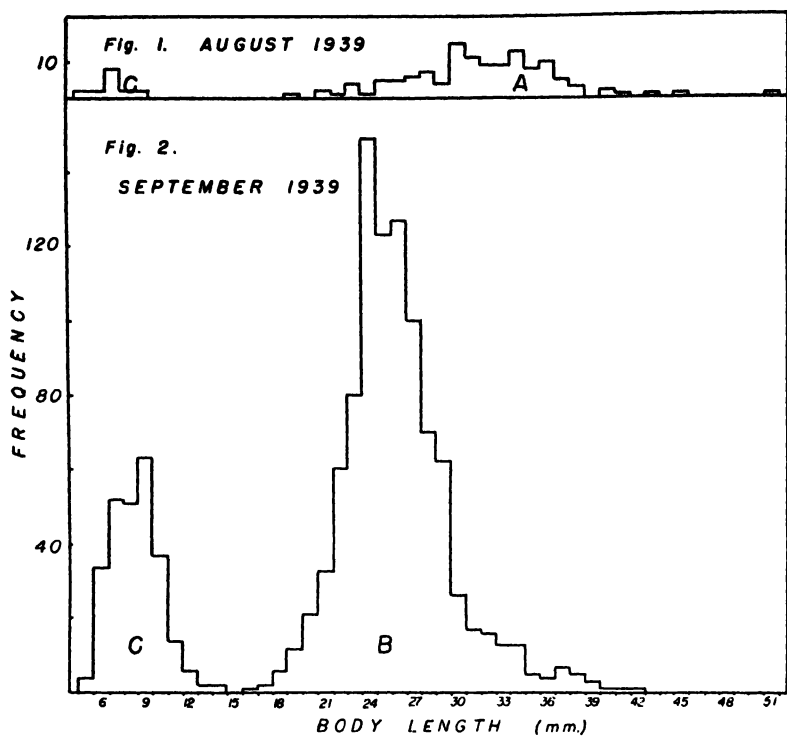


FIG. 1. Lake Harbour, 1939: July 24, August 1, 3, 5, 9, 10—141 specimens.

FIG. 2. Group B. Pangnirtung, 1939: September 13—958 specimens.

Group C. Hebron, 1939: September 18—265 specimens.

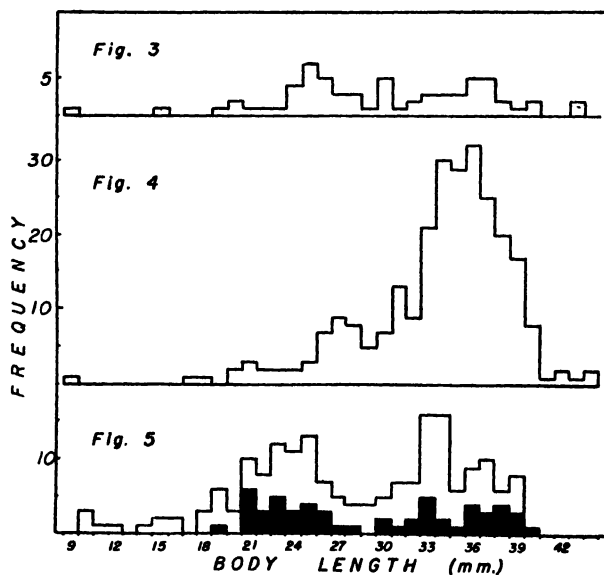


FIG. 3. Gabriel Strait, 1940: August 4 and 5—65 specimens.

FIG. 4. Frobisher Bay, 1940: August 8 and 10—263 specimens.

FIG. 5. Lake Harbour, 1940: July 30 and 31 (black) and August 14, 21, 23 (white)—187 specimens.

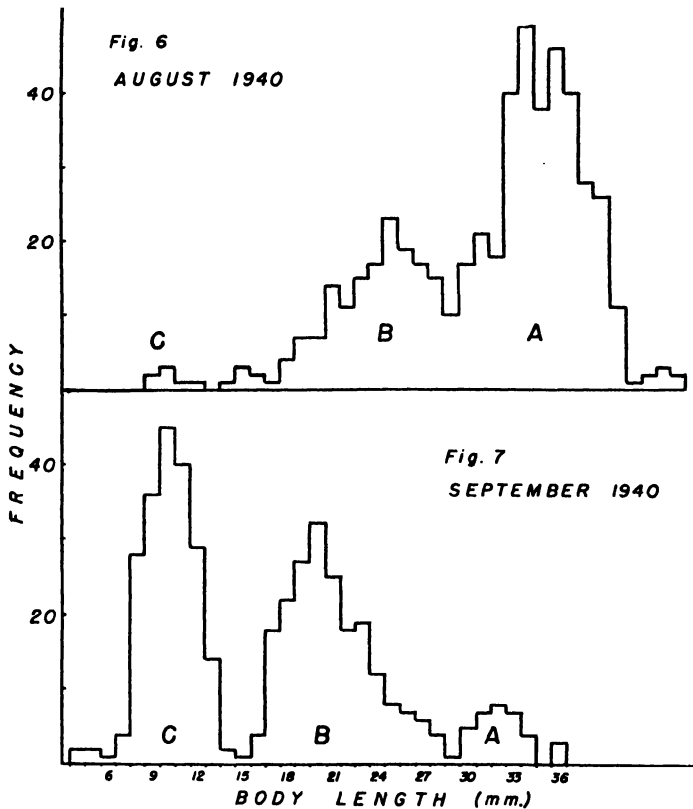


FIG. 6. Sum of Figs. 3, 4, and 5—515 specimens.

FIG. 7. Clyde River, 1940: September, 7, 8, 11, 12, 13—441 specimens.

are present in the *B* group in Fig. 2, and that the enormous predominance of the adolescent group obscures their presence. The wide range of body length covered would point to this conclusion (16 to 42 mm. in Fig. 2 as against 15 to 29 mm. in Fig. 7).

There was considerable variation in the state of the gonads in the large group, *A*, a variation that showed little correlation with length; this agrees with the findings of Kramp (7): "... The length of a mature *Sagitta elegans* is on the average 34 to 35 mm., but varies very much." The ovaries ranged from small, with no large eggs, to almost spent, with still a few ripe eggs left in the oviducts. Using Kramp's (8) system of grouping, the ovaries varied from his stage II to stage IV. The commonest was stage III, with some ripe eggs amongst the unripe ones. The testes varied in the same way, from having a considerable number of polyblasts in the tail cavities, in various stages of development, to spent (Kramp's stages II to IV). They differed markedly from the testes of the *B* group in that there were none without some polyblasts in the cavities. The seminal vesicles were developed.

The inferences are that there is a fairly long spawning period in the summer and fall, probably from June or July to October, indicated by the wide varia-

tion in the gonads of the breeding group, and that Group *B* will not spawn until the following year.

The size frequencies in Figs. 1, 2, 6, and 7 are arranged in pairs, August and September, for the two years 1939 and 1940. They are self-explanatory. The parental responsibility of Group *A* for Group *C* has been inferred from the increase in *C* and the decrease in *A* during the two months. Fig. 6 is an aggregate made up from Figs. 3, 4, and 5. It will be seen that it simply accentuates the modes already visible in the separate curves made from the collections at Gabriel Strait, Frobisher Bay, and Lake Harbour. It will also be observed that the gap of two weeks between the black and white portions of Fig. 5 has made no difference to the size of the animals or to the positions of the modes. Growth is very slow in Arctic water, even during the summer, and it is not surprising that *Sagitta* takes two years to reach maturity.

Discussion

Russell (14) records four, and possibly five broods of *Sagitta elegans* produced in one year at Plymouth. Pierce (12) found only one spawning period, extending from January to May, at Port Erin, Isle of Man, and there appeared to be only one brood involved. In the North Sea, Wimpenny (18) found two breeding periods, with indications of a third in October. Meek (11) working on *Sagitta* from Northumbrian waters, found two size groups on one occasion, and concluded that there was an overlap of breeding cycles, at least in that instance. This is the only record of such a phenomenon from British waters, or indeed in any temperate waters. The numbers measured, however, were not large. Along the Atlantic coast of North America there seems to be one long breeding period, and there is no evidence available for the production of more than one brood per year; that is, there is no evidence of the succession of broods found by Russell. Quoting from Redfield and Beale (13): "*S. elegans* appears to breed in the Gulf of Maine in the late spring and summer. Individuals less than 10 mm. in length may be taken from May to September in Massachusetts Bay and on Georges Bank. Huntsman and Reid [6] consider the spawning season in the Bay of Fundy to extend from April to September, though the eggs do not develop properly until late summer, and the authors doubt if conditions are sufficiently favorable in that locality to enable the species to perpetuate itself except by immigration. In the Woods Hole region Fish [4] found ripe eggs as early as March and April."

In Arctic waters, Ussing (17) and Kramp (7 and 8) agree that there is only one annual generation, but there seems to be some variation in the time of the breeding period. Ussing, in east Greenland, found it to begin at the end of February, and to extend possibly to May. Kramp (7) concluded that the bulk of the population in west Greenland breed in the late autumn, but that breeding may start some time before. Working on the material of the "Godthaab" expedition he came to the conclusion (8) that the breeding extends from May to September, a conclusion not very different from that reached in the present work.

There are indications, therefore, of a gradient of breeding habit from the condition found in the English Channel by Russell (14), with several broods produced in the year, through the one-brood-per-year conditions that apparently exist in the Gulf of Maine and the Gulf of St. Lawrence (6), to the situation in the Arctic, where not only is there no more than one brood produced each year, but each brood takes two years to mature.

A comparison of the results described in this paper with those obtained from Disko Bay, west Greenland (Dunbar (3)) is interesting. The *Sagitta* from Disko Bay showed a bimodal size grouping, the ranges of the two groups along the horizontal axis being 8 to 21 mm. and 21 to 43 mm., respectively; the mode of the larger group was approximately at 31 mm. The Disko Bay material was collected on July 31, and August 5 and 20, in 1936. This distribution is in some contrast with the August conditions in Baffin Island. It is somewhat more like the September situation shown in Fig. 2, but the position of the mode in the larger group is markedly different. The matter does not allow much discussion, because of lack of data from Greenland, but there would seem to be fertile ground here for further investigation. One fact of doubtless major importance is that the temperatures in Disko Bay were somewhat higher than in Baffin Island. Negative temperatures were not met with, and the temperature range, between 10 and 100 metres, was from 0.16° to 2.55° C. The epiplankton was also considerably different, and though Atlantic water was not demonstrable hydrographically, the plankton was suggestive of its presence.

In addition to the *Sagitta* population in Disko Bay, two euphausiids, *Thysanoessa inermis* Krøyer and *T. raschii* M. Sars, showed the same type of size grouping. Bimodal distributions, representing separate year groups, have also been found by Stephensen (16), in *Anonyx nugax* Phipps from east Greenland, and by Ruud (15), in *Euphausia superba* Dana from the Antarctic. Such alternating cycles in the plankton, involving the simultaneous existence of two generations separated in age by at least one year, are not found elsewhere than in polar waters, with the possible exception mentioned above (11). They are evidently effective in ensuring large numbers of the species under conditions of very slow growth. If they are to be looked upon as adaptations in the Darwinian sense, then the advantage conferred thereby on the species must be realized by the ability of each brood to overflow on occasion, and in part, into the next year, so that the failure of one brood may be made good by the development of a sufficiently large lag in the breeding cycle of the other. The small seasonal variation in temperature in polar water would favour such a distortion of phase, and indeed a tendency to go out of phase in this manner is the only way in which genetic contact between the broods could be maintained. Lacking this, the two broods would be entirely separated, and the conditions would make possible a differentiation that might in time reach specific proportions. In short, the two-phase breeding cycle offers yet another form of isolation conducive to speciation. It should be noted, in

conclusion, that the effectiveness of such an isolation depends on the occurrence of only one breeding period per individual.

Acknowledgment

During the collection of the material for this and other work, the author was a member of the Canadian eastern Arctic Patrols of 1939 and 1940. He wishes to express his gratitude to the Bureau of Northwest Territories and Yukon Affairs for the opportunity to make these studies.

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METHOD OF ESTIMATING THE POPULATION OF AN AGRICULTURAL PEST OVER AREAS OF MANY SQUARE MILES¹

BY GEOFFREY BEALL²

Abstract

An investigation was undertaken to determine how the number of insects on a crop can be estimated most accurately by sampling when the position and number of the fields involved are initially unknown. The insect considered was the Colorado potato beetle, *Leptinotarsa decemlineata* Say, on potato crops in Caradoc township, Middlesex county, Ontario. A sample was obtained by examining a randomly chosen fraction, .001, of each field in a random selection of the blocks into which the township is divided by roads. The estimate formed on the basis of such procedure would have been somewhat better if less work had been done within each field and more blocks had been covered. Uniform sampling in all fields would have given almost as good an estimate as the proportional method employed.

1. Introduction

Entomologists are frequently concerned with the population of an insect in fields under a certain crop over a region many square miles in extent, when the position and number of the fields concerned are initially unknown. It is desired to find how accurately the total number of insects and the average number per unit area can be estimated under such conditions by sampling. The insect considered, as an example, was the Colorado potato beetle, *Leptinotarsa decemlineata* Say (larval and imaginal stages), as it occurred on July 13, 1937, in the potato fields of an area of 59 square miles in the northern part of the township of Caradoc in the county of Middlesex, Ontario.

2. The Estimation of Total Population and the Variability of that Estimate

Inasmuch as, in an investigation of this nature, it will generally be impossible to examine all the fields included in the area subject to survey, the first necessity is to secure a representative selection of fields whose number and location are generally unknown. Since, however, it will be impossible forthwith to choose randomly from among the fields, of which the identity is unknown, it appears that one might best, in a township divided by roads into small known blocks, choose such blocks at random and examine all fields under a given crop in the chosen blocks. In this form the problem is clearly analogous to that of Neyman (3) when he sampled for households from the statistical districts of the Polish census and considered all the men in a house. The part of Caradoc township chosen for the survey was broken by a rectangular

¹ Manuscript received May 23, 1941.

Contribution No. 2075, Division of Entomology, Science Service, Department of Agriculture, Ottawa, Canada.

Presented at the ecological session of the Biometrics Section of the American Statistical Association in Chicago, Ill., on December 26, 1940.

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TABLE I

RESULTS FROM THE SURVEY OF PART OF CARADOC TOWNSHIP FOR POTATO BEETLES

Block, h	Field, i	Size of field, N_{hi}	Units examined, n_{hi}	Insects found, $\sum_{k=1}^{n_{hi}} X_{hik}$	Estimated population, F_{hi}	Estimate, s_{hi}^2
1	1	4466	4	186	207,669	1216.33
	2	2072	2	1	1036	0.50
	3	3483	3	0	0	0.00
	4	6573	6	116	127,078	343.07
	5	1173	2	0	0	0.00
	6	3630	4	31	28,132	124.25
	7	2816	3	70	65,707	917.33
	8	2294	2	99	113,553	544.50
	9	10270	10	8	8216	6.40
	10	3971	5	2	1588	0.80
2	1	4933	5	43	42,424	124.30
	2	6800	7	2	1943	0.57
	3	583	2	0	0	0.00
	4	16609	15	0	0	0.00
3	1	7280	7	10	10,400	14.29
	2	5964	6	10	9940	130.67
	3	3373	3	0	0	0.00
	4	2053	2	0	0	0.00
	5	5680	6	0	0	0.00
	6	15166	15	50	50,553	122.10
	7	17270	17	0	0	0.00
4	1	16950	17	0	0	0.00
	2	6517	7	0	0	0.00
	3	2500	2	0	0	0.00
	4	1536	2	0	0	0.00
	5	3233	3	9	9699	13.00
	6	1176	2	27	15,876	40.50
	7	4620	5	0	0	0.00
	8	8217	8	86	88,333	505.07
	9	3103	3	0	0	0.00
	10	2460	3	0	0	0.00
5	1	6380	6	0	0	0.00
	2	2000	2	0	0	0.00
	3	5372	6	0	0	0.00
	4	1020	2	44	7140	18.00
	5	5676	6	0	0	0.00
	6	6512	6	0	0	0.00
	7	1458	2	0	0	0.00
	8	5867	3	25	48,892	17.33
	9	8436	8	0	0	0.00
	10	2970	3	0	0	0.00
	11	10878	10	0	0	0.00
6	1	8256	6	134	184,384	2349.07
	2	10577	6	32	56,411	124.27
	3	4650	5	25	23,250	125.00
	4	5647	6	53	49,881	188.17
	5	1120	2	45	25,200	264.50
	6	3540	4	4	3540	4.00
	7	5640	6	52	48,880	175.07
	8	1232	2	0	0	0.00
	9	5802	6	121	117,007	748.97
	10	2800	3	0	0	0.00
	11	1689	2	15	12,668	112.50

TABLE I—*Concluded*RESULTS FROM THE SURVEY OF PART OF CARADOC TOWNSHIP FOR POTATO BEETLES—*Concluded*

Block, h	Field, i	Size of field, N_{hi}	Units examined, n_{hi}	Insects found, $\sum_{k=1}^{n_{hi}} X_{hik}$	Estimated population, F_{hi}	Estimate, s_{hi}^2
7	1	1911	2	0	0	0.00
	2	3306	3	0	0	0.00
	3	3870	4	12	11,610	36.00
	4	1892	2	0	0	0.00
	5	1700	2	73	62,050	312.50
	6	1380	2	36	24,840	392.00
	7	4800	5	74	71,040	102.13
	8	5742	6	93	89,001	702.70
	9	3882	4	152	147,516	750.00
	10	2053	2	0	0	0.00
	11	1009	2	15	7568	112.50
8	1	8246	8	42	43,292	114.50
	2	15407	14	0	0	0.00
	3	3888	5	0	0	0.00
	4	1440	2	16	11,520	128.00
	5	7546	2	22	83,006	72.00
	6	1413	2	0	0	0.00
	7	9667	10	348	336,412	1051.29
	8	1530	2	0	0	0.00
	9	971	2	1	486	0.50
	10	2628	3	9	7884	10.80
	11	3333	3	126	139,986	769.00

system of roads into $M = 41$ blocks typically of 1.26 square miles. From these blocks a sample of $m = 8$ blocks was chosen randomly for examination.

Within the eight blocks drawn, 73 commercial fields of potatoes were found and all were examined. Within the h^{th} ($h = 1 \dots M$) block say there were C_h fields of potatoes and each of these fields was examined. In the same way, however, that it was impracticable to examine all blocks and therefore was necessary to sample so it became again necessary to carry out further sampling within the fields*. In this feature the present problem differs from that of Neyman for he was not forced to sample within his man found in a sample household. The material within the fields was secured at sampling positions randomly chosen in terms of rows from the edge of the field and paces from the end of the field along the rows. At these positions, the men making the examination laid down in the direction of the rows a light rod 3 yd. long and examined the potatoes of one row against which the rod lay. These stretches of three yards of potatoes constituted sampling-units, or the independently placed elements of sampling. From the numerical results of the work, as shown in Table I, it is required first to form an estimate of the total population of potato beetles in the region.

* The fields might have been subdivided into parts, such as quadrants, to be subjected to independent sampling but the work proved sufficiently complex without this refinement.

Consider now the population, X_{hi} , of insects on the hi^{th} ($i = 1 \dots C_h$) field, consisting of N_{hi} sampling-units from which n_{hi} sampling-units were examined. Let the number of insects on the hik^{th} ($k = 1 \dots N_{hi}$) sampling-unit be X_{hik} .

$$F_{hi} = \frac{N_{hi}}{n_{hi}} \sum_{k=1}^{n_{hi}} X_{hik} \quad (1)$$

where F_{hi} is simply the population of a field as it is commonly estimated, i.e., the number of insects found is divided by the fraction of the field examined. From Equation (1) the estimate of the population, X_h , of insects on the h^{th} block is

$$F_h = \sum_{i=1}^{C_h} F_{hi} \quad (2)$$

i.e., the population on a block is estimated by adding the estimates for the various fields within the block. Having made, as in Equation (2), an estimate of the population in each block there must be again formed an estimate of the number, X , of insects over the whole region from a sample of blocks, as

$$F = \frac{M}{m} \sum_{h=1}^m F_h \quad (3)$$

where F is formed in the same way as F_{hi} , i.e., the number of insects estimated to have been found on the blocks examined is divided by the fraction of the blocks examined. In connection with Equation (3) it is reassuring to note that Neyman (3, p. 579) makes it clear that F is a true estimate, i.e., its expectation coincides with the true population of insects, whether or not the blocks are of a constant magnitude.

The estimate, F_{hi} , of the number of insects in each field examined is shown in Table I, together with the data, i.e., N_{hi} , n_{hi} , and $\sum_{k=1}^{n_{hi}} X_{hik}$, on which the estimate was based. Following Equation (3), remembering for the present case that $M = 41$ and $m = 8$ and calculating from Table I it may be seen that $F = 12,280,000$. In Table I there is added a column of values, s_h^2 , which are discussed in Section 3.

The chance variation of the estimate, F , is measured by the standard deviation, σ_F , which may, following Neyman (3), be estimated as

$$s_F = \left[\{ M(M-m) s_h^2 \} / m \right]^{\frac{1}{2}} \quad (4)$$

where

$$s_h^2 = \frac{1}{m-1} \left\{ \sum_{h=1}^m F_h^2 - \frac{1}{m} \left(\sum_{h=1}^m F_h \right)^2 \right\} \quad (5)$$

For the present case, $s_F = 3,267,000$.

3. The Improvement of Estimation by Varying the Closeness with which Fields were Examined

From the results of Section 2, the ratio, $s_F/F = 0.27$, so that the estimate, F , is not very reliable. Such a result gives point to the desirability of reducing the variability of the estimate by some change in field method. With

such a purpose in view, consider the possibility of having examined perhaps more blocks and done less work of sampling in the fields of those blocks, or vice versa, and also the effect of such a change on the magnitude of σ_F .

A primary consideration in the comparison of results from any proposed rearrangement of procedure is that the totality of work, W , involved should be unchanged. Obviously,

$$W = \frac{m}{M} W_M + \frac{mn}{M} W_N \quad (6)$$

where W_M is the work, such as passing from block to block and from field to field within a block, of examining all possible blocks; where W_N is the remaining work within fields, i.e., that of counting the insects on all the sampling-units of all the fields of all the blocks; and where, as was approximately the case for the present study,

$$n = \frac{n_{hi}}{N_{hi}} \quad (7)$$

i.e., the number of sampling-units examined in a field was proportional to the size of the field. Equation (6) simply supposes that if the fraction sampled in a field is held constant, the total work of a survey must vary approximately as the number of blocks examined or, if the number of blocks is held constant, the total work must vary approximately as the fraction sampled in each field.

Since the chance variation of the grand estimate, F , arises from variability between blocks and variability within fields, it may be studied by breaking σ_F in two parts, i.e., writing

$$\sigma_F^2 = \sigma_{F'}^2 + \frac{M}{m} \sum_{h=1}^M \sigma_{F_h}^2 \quad (8)$$

where the squared standard deviation of the grand estimate F' , if it were based on an exhaustive examination within the fields of the m blocks touched, has been termed $\sigma_{F'}^2$ and the squared standard deviation of the estimate, F_h , which arises from sampling within the fields of the h th block, $\sigma_{F_h}^2$. Now, after Beall (1)

$$\sigma_{F_h}^2 = \sum_{i=1}^{C_h} \frac{N_{hi}(N_{hi} - n_{hi})}{n_{hi}} \sigma_{hi}^2 \quad (9)$$

where the chance squared standard deviation of sampling-units within the hi th field is

$$\sigma_{hi}^2 = \left[\sum_{k=1}^{N_{hi}} \left\{ X_{hik} - \left(\sum_{k=1}^{N_{hi}} X_{hik} \right) / N_{hi} \right\}^2 \right] / (N_{hi} - 1) \quad (10)$$

and so from Equations (4), (8), and (9),

$$\sigma_F^2 = \frac{M(M-m)}{m} \sigma_{F'}^2 + \frac{M}{m} \sum_{h=1}^M \sum_{i=1}^{C_h} \frac{N_{hi}(N_{hi} - n_{hi})}{n_{hi}} \sigma_{hi}^2 \quad (11)$$

where

$$\sigma_{F'}^2 = \frac{1}{M-1} \sum_{h=1}^M \left(X_h - \frac{1}{M} \sum_{h=1}^M X_h \right)^2 \quad (12)$$

Under the circumstances of Equation (7), from Equation (11)

$$\sigma_F^2 \approx \frac{M(M-m)}{m} \sigma_h^2 + \frac{M}{m} \frac{(1-n)}{n} \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi} \sigma_{hi}^2 \quad (13)$$

In Equation (13) it may be seen how the variability of the estimated population, F , depends upon two sources of variability in the population: first, upon the variability, σ_h^2 between blocks quite apart from any sampling within fields and, second, upon the variability within fields in the form indicated in the second term.

Now consider when σ_F will be minimal, i.e., when F will have the smallest variability or when X will be estimated with the greatest accuracy, as more or less of the time is spent in sampling fields. From Equations (6) and (13) σ_F is minimal when

$$n = \left\{ \left(W_M \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi} \sigma_{hi}^2 \right) / \left(W_N M \sigma_h^2 - W_N \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi} \sigma_{hi}^2 \right) \right\}^{\frac{1}{2}} \quad (14)$$

It may be noted in Equation (14) that the value of n indicated is independent of the total amount, W , of work that may be done.

In the present study, it was quite impossible to make a complete count over the region under consideration in order subsequently to demonstrate the results to be obtained from sampling. Accordingly, since knowledge of the situation was necessarily limited to the various estimated values found by sampling, the discussion will proceed on the basis of using these estimated values as if they were true population values; it is realized, of course, that the population under discussion is not the one that actually existed in Caradoc but it is felt that the artificial population is very similar to the true, has the same characteristics, and might easily have existed. Accordingly the estimates s_{hi}^2 , shown in Table I, will be taken as values, σ_{hi}^2 , and from them will again be found

$$\frac{M}{m} \sum_{h=1}^M \sum_{i=1}^{C_h} \frac{N_{hi}(N_{hi} - n_{hi})}{n_{hi}} \sigma_{hi}^2$$

to get an estimate of

$$\sum_{h=1}^M \sum_{i=1}^{C_h} \frac{N_{hi}(N_{hi} - n_{hi})}{n_{hi}} \sigma_{hi}^2$$

as 413,840,000,000 from which value, used with the estimate of σ_F^2 in Equation (11) there is obtained an indirect estimate of the squared standard deviation, $\sigma_h^2 = 50,590,000,000$, for blocks subject to complete examination. In the study in Caradoc township, a record was kept of the work, in man-minutes, required for the various operations of the examination, that of sampling for blocks was $W_m = 1785$ and that of sampling within fields was $W_n = 1257$, so that one estimates $W_M = (41/8) W_m = 9148$ and since approximately $n_{hi} = 0.001N_{hi}$, $W_N = (41/8) (1000) W_n = 6,442,000$.

Using the above estimates as parameters of a population from Equation (14) $n = n_{hi}/N_{hi} = 0.000,456$ makes σ_F minimal, for all values of W . If $W = 3042$, from Equation (6) the corresponding value of m would be 10.3 blocks. In Table II there is shown for values of $m = 5 \dots 13$, when $W = 3042$, the

TABLE II

THE MAGNITUDE OF σ_F AND THE LEVEL OF SAMPLING IN FIELDS WHEN TWO AMOUNTS OF WORK MAY BE DONE IN TWO WAYS AND VARIOUS NUMBERS OF BLOCKS USED

m	$W = 3042$		$W = 6084$		$W = 3042$		$W = 6084$	
	$n_{hi} \propto N_{hi}$	$\sigma_F + 1000$	$n_{hi} \propto N_{hi}$	$\sigma_F + 1000$	$n_{hi} \text{ constant}$	$\sigma_F + 1000$	$n_{hi} \text{ constant}$	$\sigma_F + 1000$
5	0.002,45	4019	0.006,32	3925	12.2	4080	31.4	3949
6	0.001,81	3671	0.005,03	3548	9.0	3747	25.0	3577
7	0.001,35	3414	0.004,11	3254	6.7	3508	20.4	3287
8	0.001,00	3228	0.003,42	3017	5.0	3344	17.0	3054
9	0.000,73	3105	0.002,88	2819	3.6	3251	14.3	2861
10	0.000,52	3054	0.002,45	2653	2.6	3240	12.2	2699
11	0.000,34	3107	0.002,10	2510	1.7	3357	10.4	2563
12	0.000,19	3384	0.001,81	2388	1.0	3749	9.0	2446
13	0.000,07	4590	0.001,56	2281	0.3	5274	7.7	2346
14			0.001,35	2189			6.7	2262
15			0.001,16	2110			5.8	2191
16			0.001,00	2043			5.0	2134
17			0.000,86	1988			4.3	2090
18			0.000,73	1946			3.6	2061
19			0.000,62	1917			3.1	2047
20			0.000,52	1904			2.6	2052
21			0.000,42	1911			2.1	2082
22			0.000,34	1946			1.7	2144
23			0.000,26	2022			1.3	2256

value of n from Equation (6), neglecting considerations of the integrality of n_{hi} , and also the value of σ_F from an Equation (13). From Equation (6), $n = 0$, when $m = 13.6$ blocks, so that 14 blocks would have been impossible, i.e., if an attempt had been made to have covered 14 blocks, no time would have been left for the sampling of fields.

If instead of making the total amount of work, $W = 3042$ man-minutes, one had made $W = 6084$, from Equation (6), $n = 0$ if $m = 27.3$ and σ_F is minimal with $m = 20.6$ blocks. In Table II the values of n and of σ_F obtaining for various values of $m = 5 \dots \dots \dots 23$ are also shown.

From the left-hand half of Table II, which concerns the present section (the other half concerns Section 4), two conclusions may be drawn: first, for $W = 3042$, the minimal σ_F was little smaller than the value found by the arrangement of work actually employed, so that arrangement was fairly satisfactory; second, the magnitude of σ_F does not change rapidly with changes in m , so that good results would have been obtained anywhere in the region of the best m , so long as the number of sampling-units examined in each field was kept low and many blocks could be covered. It is of interest to note, in the present case where the universe of blocks is very small, that the reduction of σ_F^2 with an increase of work is rather sharp, thus when W is increased from 3042 to 6084, σ_F^2 is reduced 61%; such reduction is maximal for the n of Equation (14).

The following question may be raised: how would the conclusions concerning the best apportionment of work have been modified if the area under survey and the work done had been q times greater than in the present case? If the variability, σ'_k , between blocks (with complete examination) and the variability within fields should be unchanged so that the factors, W_M , W_N ,

and $\sum_{k=1}^M \sum_{i=1}^{C_k} N_{ki} \sigma_{ki}^2$ in Equation (14) would be increased q times, the

value of n that makes σ_F minimal would be unaffected. Even if the extension of the territory should introduce heterogeneity but the territory were subdivided into q sections or strata* of the same magnitude and subject to the same variability between blocks and within fields as in the total area of the present work, the same value of σ_F for a given value of n should be obtained and hence the desired value of n would be unchanged. If, however, the introduction of heterogeneity should modify the situation pictured above by the enlargement of σ'_k , from Equation (14), the desired value of n would be reduced, i.e., the best procedure would be to sample within fields still more lightly than in the present case.

Under the circumstances that no heterogeneity should be introduced, or it should be controlled by stratification, and the true population should be q times greater than in the present case, from Equation (13), σ_F would be increased $q^{\frac{1}{2}}$ times so that the ratio s_F/F , mentioned at the beginning of the present section, would be only $q^{-\frac{1}{2}}$ times as great as for the present study.

4. The Variability of the Estimate when the Same Number of Sampling-units is Examined in each Field

Since it is a common practice of field men to examine a constant number, $n_{ki} = n'$, of sampling-units in each, hi^{th} , field entered, consider, for the data of the present work, how the variability, σ_F , from such a practice compares with that to be obtained from the system employed, as in connection with Equation (7), of making the number, n_{ki} , of sampling-units vary with the size, N_{ki} , of the field.

First consider the disposition of the work involved by the practice under consideration. After the manner of Equation (6), it may be written that the fixed total amount of work is

$$W \approx \frac{m}{M} W_M + \frac{n'}{N} \frac{m}{M} C W_N \quad (15)$$

using the symbols of Section 3 with $N = \sum_{k=1}^M \sum_{i=1}^{C_k} N_{ki}$, the total number of

sampling-units, and $C = \sum_{k=1}^M C_k$, the total number of fields in the region.

* No stratification was employed in the present work but for cases where it may be employed the appropriate theory is discussed by Beall (1).

Considering the nature of σ_F involved by the practice under immediate consideration from Equation (11), on substituting n' for n_{hi}

$$\sigma_F^2 = \frac{M(M-m)}{m} \sigma'^2_h + \frac{M}{m n'} \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi}^2 \sigma_{hi}^2 - \frac{M}{m} \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi} \sigma_{hi}^2 \quad (16)$$

Again consider when σ_F will be minimal for a fixed total amount, W , of work. From Equations (15) and (16), σ_F is minimal when

$$n' = \left[\left(N W_M \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi}^2 \sigma_{hi}^2 \right) / \left\{ C W_N \left(M \sigma'^2_h - \sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi} \sigma_{hi}^2 \right) \right\} \right]^{\frac{1}{2}} \quad (17)$$

where n' is independent of W .

For the present section there are needed the estimates from the data of $N = 1906,000$, $C = 384$, and $\sum_{h=1}^M \sum_{i=1}^{C_h} N_{hi}^2 \sigma_{hi}^2 = 2,538,629,000,000$.

Employing these quantities and others previously used in Section 3, from Equation (17) for $W = 3042$ the desired value is $n' = 2.94$. For this value of n' , from Equation (15) the value of $m = 9.6$ is close to the value, $m = 10.3$, previously obtained, under the condition that $n_{hi} \propto N_{hi}$, for the best value of n .

On the right-hand side of Table II there are shown for various values of m the corresponding values of n' , neglecting the consideration of integrality, from Equation (15), and of σ_F from Equation (16), for $W = 3042$ and $W = 6084$. From a comparison of these values with the corresponding values for the case where n_{hi} is proportional to N_{hi} , it can be seen that although σ_F is now somewhat greater, the system of apportioning work within fields makes little difference to the final result. The difference is, however, greatest when the sampling within fields is light and many blocks are examined. Accordingly, it is judged that the practice, as mentioned at the beginning of the present section, of examining a constant number of sampling units in each field will furnish data from which a satisfactory estimate of the total population may be made.

The possibilities of improving the estimate by modifications in the system of sampling within fields can be appreciated from the consideration of σ'_F , i.e., the value of σ_F if a complete examination ($n_{hi} = N_{hi}$) should be made within fields. Working with eight blocks from Equation (11), that $\sigma'_F = 2,925,000$ in contrast to the results of Table II, i.e., $\sigma_F = 3,228,000$ when $n_{hi} = 0.001 N_{hi}$ and $\sigma_F = 3,344,000$ when $n_{hi} = 5$. Accordingly, it does not appear that by adjustment of the apportionment in the fields there is much room for reduction in σ_F . In view of this situation further refinements of the work do not seem to be justified, in particular the possibility suggested by the work of Beall (1) of adjusting the number of sampling-units to be examined according to the variability of counts within the fields.

5. The Estimation of Infestation per Unit Area

Often the purpose in making a survey of the type carried out in Caradoc township will be to form an estimate of the intensity of infestation, say, of the number, J , of insects per sampling-unit. The problem is discussed by Neyman (3), following whom there is estimated

$$J = F/G \quad (18)$$

where

$$G = \frac{M}{m} \sum_{h=1}^m \sum_{i=1}^{c_h} N_{hi} \quad (19)$$

is an estimate of N , the total area under the crop. In Equation (18) it will be noted that the number of insects per sampling-unit is estimated as the ratio of the estimated total number of insects to the estimated total area.

Since the exact chance distribution of J is unknown, it is not possible to deal with its variability so easily as with that of F . It is possible, however, if the distributions of F and of G are known, to find approximately, by a method proposed by Geary (2), an upper and a lower limit beyond which J will fall by chance with only a prespecified degree of probability. From his discussion may be obtained values of J exceeded respectively by chance with a probability of 0.005, by finding the roots of

$$(N^2 - 6.6353\sigma_G^2)J^2 - 2(NX - 6.6353R\sigma_G\sigma_F)J + X^2 - 6.6353\sigma_F^2 = 0 \quad (20)$$

where

$$\sigma_G = [\{M(M - m) \int_h^2\} / m]^{\frac{1}{2}} \quad (21)$$

is after Neyman (3) the standard deviation of the estimate, G , where

$$\int_h^2 = \frac{1}{m-1} \sum_{h=1}^m \left\{ N_h - \frac{1}{m} \left(\sum_{h=1}^m N_h \right) \right\}^2 \quad (22)$$

and where R is the correlation between G and F .

Again, as in Sections 3 and 4, suppose one deals with a population having the parameters estimated from the field work with $X = 12,280,000$, $N = 1,906,000$, and $\sigma_F = 3,267,000$. The standard deviation of the quantities, N_h , calculated from the observations of Table I, is 11,331. Accordingly, in the case where $m = 8$, from Equation (21), $\sigma_G = 147,400$. From Table I the correlation coefficient of N_h and F_h is -0.0056 , i.e., N_h and F_h are independent and hence N and F are independent and in Equation (20), $R = 0$. Under these conditions ($W = 3042$ man-minutes and approximately $n_{hi} \propto 0.001N_{hi}$) the limits of J from Equation (20) are 11.4 and 2.0. Since it is improbable that generally in field work $R = 0$, it should be noted that with the modification to the preceding situation that $R = 0.5$, the limits become 9.8 and 1.8, and do not differ greatly from those found previously.

In Section 3 it was seen that for $W = 3042$, σ_F could be reduced from 3,267,000 to 3,054,000 by making $m = 10$ and $n_{hi} \propto N_{hi}$. The same field procedure would make the limits of J , 11.0 and 2.3, where the corresponding reduction is 0.7 in their range. If the case is modified so that $W = 6084$ and $m = 20$, the limits are 8.4 and 4.1. From this result it can be seen that

as in the case of estimating the total population, so in the case of estimating infestation per unit area, an appreciable improvement in the estimate can be made only by increasing the amount of work.

6. Implications of the Study for Routine Practice

The present study should furnish an example of the manner in which surveys of the number of insects occurring on a cultivated plant, of which the position and number of fields is unknown, may be made. The variability of the estimates made in the present study are disconcertingly high; nevertheless, the procedure of random sampling for blocks and the random sampling of fields within those blocks still appears to be the best practical method for field problems such as that considered in the present work.

The fraction of the total available time that should be spent in sampling within fields was dependent on the variability between blocks, the variability within fields, and the time required to make a complete count of blocks or within fields. It is impossible to judge how general the relative magnitude of these quantities, as estimated in the present work, will prove for other material. For the present case it is, however, very clear that it was desirable to spend the time available for the survey mainly in covering as many blocks as possible and to sample within fields very lightly. While the estimate, F , proved better when made on the basis of varying the amount of sampling in a field with the size of the field, rather than on the basis of sampling equally in all fields, if, as the writer suspects, field men will find it a practical convenience to employ the second procedure it will be followed by little deterioration of the estimate, F .

In the present work the variability of the estimate of total population of potato beetles was high relative to the estimate and the estimate of population per unit area was very variable. There appears to have been no way of improving these estimates markedly, short of spending more time in the field. In so far, however, as the variability of the estimate, F , was due mainly to the variability between blocks, in such work as the present every effort should be made to control the latter variability by means of stratification, as mentioned at the end of Section 3, of the area.

7. Acknowledgments

The author was aided in the field work of this study by Dr. G. M. Stirrett and Messrs. H. F. Hudson and A. A. Wood of the Dominion Entomological Branch. Dr. J. W. Hopkins and Dr. W. R. Thompson made helpful criticisms of the discussion.

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METHODS FOR THE INVESTIGATION OF RANCIDITY, THEIR INTERRELATION, AND APPLICATION TO BACON FAT¹

BY W. HAROLD WHITE²

Abstract

A procedure for the preparation of bacon fat for physical and chemical examination is described. The relative suitability of the determination of peroxide oxygen, free fatty acid, Kreis and aldehyde values, the content and acidity of water-soluble materials, and the oxidation-reduction potential for the objective estimation of rancidity was studied using samples of Wiltshire bacon fat obtained from 22 Canadian packing factories. Other measurements related to quality, and possibly also to spoilage, were made. These included a determination of the hardness, iodine number, melting point, refractive index, and the contents of moisture and of fat. Modifications of certain of the experimental procedures are outlined.

The computation of simple coefficients of correlation between the measured properties showed the following to be related: peroxide oxygen content with each of the Kreis and aldehyde values, the acid value of the water-soluble materials and the oxidation-reduction potential; the iodine number with the refractive index; and the refractive index with the contents of moisture and of free fatty acid. The peroxide oxygen and Kreis tests are considered to be the most suitable for the estimation of rancidity.

The relative tendency for bacon sides to become rancid was found to be primarily an inherent characteristic of the fat of a particular side, and showed little relation to known variations in either the processing factors or the properties of the lean meat. Observed variations in the quality of the fat were due mainly to differences between sides, but also in part to non-uniform selection and grading in the various factories.

Introduction

The results of an extensive survey on the chemical, bacteriological, and physical properties of Canadian Wiltshire bacon have been reported in previous publications from these laboratories (4, 6-8, 14, 15, 35, 36, 38-42). In addition to the various measurements on the lean meat, studies were also made on the fat with respect to its quality and, in particular, to the development of rancidity. The purpose of the present paper is to present the results of investigations on methods of estimating rancidity and quality in bacon fat, the interrelation of the various properties, and the relative importance of the sources contributing to their variation in Canadian Wiltshire bacon fat.

Methods

The absolute estimation of the extent of development of rancidity in fats by physical or chemical procedures is difficult because of the complex nature of the reactions associated with spoilage and their dependence on a number of accessory factors, such as composition of the fat (2), exposure to heat and

¹ Manuscript received June 30, 1941.

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Material selected from a thesis presented to the University of Western Ontario in partial fulfilment for the degree of Master of Arts. Issued as Paper No. 70 of the Canadian Committee on Storage and Transport of Food and as N.R.C. No. 1007.

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light (2, 3), availability of oxygen (21), and the magnitude of the exposed surface (27). Although a variety of methods have been proposed, odour and taste are still generally considered to be the final and most reliable criteria of rancidity. However, chemical and physical methods are at least of value for comparisons between similar fats treated in a comparable manner.

In view of the apparent uncertainty concerning the relative merits of the various objective measurements for rancidity, a number of those considered as being most suitable were selected for study. These included determinations of peroxide oxygen, free fatty acid, Kreis value, aldehydes, water-soluble compounds, and the oxidation-reduction potential. In addition the following measurements, related particularly to the quality of the fat, but in certain instances also to spoilage, were made: hardness, iodine number, melting point, refractive index, and the moisture and total fat contents.

Preparation and Extraction of the Sample for Analysis

Since bacon fat is relatively unstable, it is essential that the preparation and extraction of the fat for chemical analysis be carried out under conditions that cause a minimum of change. Three steps are involved, namely, the fine subdivision of the sample to facilitate the subsequent steps of drying and extraction.

It was found that subdivision could be best obtained by finely chopping the fat, while in the frozen condition, with a sharp knife. Although the possibility of the introduction of small particles of metal existed, this was of little importance, since the extracted material was not to be used for comparative stability tests.

It was considered that the fat could be most conveniently and efficiently dried, either by heating *in vacuo* or by mixing thoroughly with an anhydrous salt, e.g., sodium sulphate. In a preliminary study, the relative efficacy of these two treatments was determined by direct comparison on separate portions of the same sample of chopped fat. In one procedure, the sample was dried *in vacuo* at 50° C. for 48 hr., and extracted in a Soxhlet apparatus with petroleum ether for three hours. By the second method, simultaneous drying and extraction were effected by mixing the chopped fat with 50% by weight of anhydrous sodium sulphate, placing the mixture in an amber glass container, adding petroleum ether and allowing the mixture to stand in the dark for 48 hr. In both instances most of the petroleum ether (a redistilled fraction boiling below 50° C.) was distilled off, and final traces were removed by heating *in vacuo* at 50° C. for approximately 16 hr. From data for the melting point, refractive index, and colour of the two samples of fat, it was concluded that the rather rigorous vacuum-drying procedure described above caused the loss of the more volatile constituents of the fat, and possibly, decomposition of certain constituents. It was consequently considered to be unsatisfactory. The simultaneous drying and extraction procedure was likewise unsuitable because of a poor yield.

In a second series of experiments, samples prepared by drying *in vacuo* at 25° C. for 16 hr., followed by extraction in a Soxhlet unit for three hours, were compared with those obtained by mixing chopped fat with 50% by weight of anhydrous sodium sulphate, followed by Soxhlet extraction. In both instances the major portion of the ether was distilled off (accelerated by aeration with nitrogen), and final traces were removed by heating *in vacuo* at 40° C. for periods of not greater than one hour. Examination of the properties of the samples showed that, although they were approximately identical, there was less possibility of oxidation, as indicated by lower values of peroxide oxygen, in the procedure utilizing sodium sulphate as the drying agent. This method has been used successfully in this investigation and others carried out in these laboratories during the past three years.

Peroxide Oxygen Content

Of the several types of methods proposed for determining peroxides in fats (12, 24, 43), the modification of Lea's iodometric procedure (24) suggested by French *et al.* (13) was selected as being the most suitable, since it gave results comparable to those by Lea's method and was simpler experimentally. *

In a previous study on chicken fat, it was shown that the observed peroxide oxygen content was approximately 20% less than the actual because of sorption of iodine by the fat (5). Since pork and bacon fat have normally a lower iodine number than chicken fat, the correction factor would probably be somewhat less than 20%.

Since nitrite, possibly present in bacon fat, interferes with the determination of peroxide oxygen, it was of importance to determine whether this compound interfered when the samples of extracted fat were prepared as described previously. Tests for nitrite (34) in a number of samples of extracted bacon fat, prepared as described previously, were negative, and indicated that precautions for its removal were unnecessary.

The values of peroxide oxygen obtained in this and other investigations carried out in these laboratories have been reported as millilitres of 0.002 *N* sodium thiosulphate, thus conforming to Lea's original practice (24). Although a variety of other methods has been suggested for their expression, this method is considered satisfactory, since the determination itself is somewhat arbitrary in nature, and of greatest value in comparisons of similar fats treated in a comparable manner.

Kreis Test

Of the original procedure (22) and the several subsequent modifications of the Kreis test, one (33) was selected as being most suitable because of its simplicity and the fact that the colour is developed in a single phase system. Investigations of the method showed it to be extremely sensitive. However, the colour intensity was proportionate to the concentration of fat used, increasing with decrease in the concentration of the fat (Table I). The Evelyn photoelectric colorimeter (10), provided with a No. 540 Rubicon

filter, was found to serve quite effectively for the measurement of colour intensity.

TABLE I

EFFECT OF VARIATIONS IN THE CONCENTRATION OF A SAMPLE OF BACON FAT ON ITS KREIS VALUE

Sample 1		Sample 2		Sample 3	
Weight of fat, gm.	Kreis value ¹	Weight of fat, gm.	Kreis value ¹	Weight of fat, gm.	Kreis value ¹
0.490	76.8	0.574	69.4	0.647	62.4
0.429	81.5	0.562	66.9	0.133	109
0.060	133	0.066	141	0.075	132
0.056	132	0.064	138	0.065	137

¹Computed as described in the text.

Since the intensity of the colour developed in the procedure follows the Lambert-Beer law, the equation $E = \log_{10} \frac{I_0}{I} / 1c$, may be reduced, when the Evelyn photoelectric colorimeter is used, to the form $E_1 = \frac{2 - \log G}{C}$, where

G is the galvanometer reading and C is the concentration of fat in grams per millilitre of final solution. Although the value of the modified extraction coefficient, E_1 , obtained in this manner has little physical significance, it is quite suitable for comparisons between different samples of fats.

Aldehydes

Aldehydes in fats have been determined by the Schiff reaction (11) and a modification of it (30). However, the Schiff reaction was considered unsatisfactory because of the distribution of the dye between two phases, the difficulty of quantitatively determining the intensity of the colour, and variations in the nature of the colour with different aldehydes. The modified test is apparently specific only for aldehydes of high molecular weight, such as glyceride-aldehyde residues (30).

A more suitable method, in that it gives a measure of the simple aldehydes of medium molecular weight, is based on the formation of the aldehyde-bisulphite addition product (26). Investigation of this procedure indicated that the 15 ml. of extract required for the determination could be more readily obtained if 25 ml., rather than the recommended 20 ml., of bisulphite solution were used. Moreover, previous observations concerning the indefiniteness and instability of the end point were confirmed (17, 23). Although the suggested use of 0.02 N iodine rather than 0.002 N (17) in the back titration of the combined bisulphite overcame these difficulties, the sensitivity of the

method was considerably reduced. It was found that a 0.01 *N* solution of iodine gave a satisfactory end point, and greater sensitivity.

Oxidation-Reduction Potential

Because of the nature of the reactions causing rancidity in fats, it was considered that the oxidation-reduction potential should be related to fat spoilage. Previous published studies appear to have been limited to milk (32). In the present investigation measurements were made by means of a Beckman pH meter, essentially a vacuum tube voltmeter, of the potential developed between a saturated calomel half-cell and a bright platinum electrode. Because of the apparent difficulties involved in the direct measurement of the oxidation-reduction potential of the fat in its solid state, a number of fat solvents were investigated as to their suitability. These included petroleum ether, benzene, and carbon tetrachloride. The oxidation-reduction potentials of bacon fats, both high and low in peroxide oxygen content, were determined when dissolved in the various solvents.

It was observed in all instances that a period of 15 min. or longer was required before the readings became relatively constant. Both the absolute magnitude of the potential for any one fat, and the differences in oxidation-reduction potential between a fat of high and one of low peroxide oxygen content varied with the solvent. Higher positive potentials were obtained for fats with greater peroxide oxygen contents than for those containing little. Carbon tetrachloride appeared to be the most suitable solvent. It gave reproducible results, could be readily obtained in a pure form, and was relatively inert to oxidation.

The following procedure was adopted as a result of these observations. One millilitre of extracted fat was dissolved in five millilitres of carbon tetrachloride. After rinsing both electrodes with carbon tetrachloride, the platinum electrode was flamed, and both electrodes rinsed with the fat-solvent solution. Readings of the oxidation-reduction potential were taken in a room at 20° C. at intervals of approximately five minutes until successive values differed by not more than one to two millivolts.

Free Fatty Acid

Although colorimetric procedures (18) have been proposed for the determination of free fatty acid, direct titration with alkali would appear to be the most commonly used. Such a procedure described previously was found to give satisfactory results (25). Since it has been shown that the practice of expressing values of free fatty acid as percentage of oleic acid is satisfactory (28), the results are computed in this form in the present and other investigations carried out in these laboratories.

Water-soluble Compounds

In the original procedure and subsequent modifications, the oxidizable compounds in the steam distillate (19) or in the hot aqueous extract (23) of a fat were determined by treatment with potassium permanganate. It was

considered that a recently proposed procedure, in which the water-soluble materials were estimated by means of sodium hypochlorite, merited investigation (31).

The procedure employed here was essentially the same as that described previously (31). However, hot aqueous extraction of the fat rather than a steam distillation was made, since the available information would indicate this to be more suitable.

The following procedure was employed in the preparation of the extract. Ten grams of finely chopped fat, weighed into a 250 ml. glass-stoppered flask, was treated with 50 ml. of boiling distilled water, the flask filled with nitrogen and stoppered. The extract was shaken slowly for two hours on a shaking machine equipped with a steam chest, then was removed, centrifuged, filtered through a moistened filter paper (Whatman No. 42, 11 cm.) into a 50 ml. volumetric flask, and, after cooling to room temperature, made up to volume.

Studies of the method, in which the quantities of extract and hypochlorite solution were varied, showed that the extent of oxidation of the materials in the extract increased with increase in the relative concentration of hypochlorite to the extract (Table II). The results obtained for Sample 4 would indicate that this was not due to a dilution effect, but depended on the actual amount of oxidizable material present.

TABLE II

EFFECT OF THE PROPORTION OF AQUEOUS EXTRACT TO SODIUM HYPOCHLORITE
ON THE OXIDATION NUMBER OF BACON FAT

Sample	Weight, gm.	Quantity of extract, ml.	Quantity 0.1 N sodium hypochlorite, ml.	Oxidation value ¹
1	10	10	15	63.7
	10	10	25	105
	10	5	25	214
2	10	10	15	49.2
	10	10	25	101
3	10	1	50	502
	10	2	50	439
	10	5	25	201
	10	10	25	103
	10	10	15	52.6
4	4.5	10	25	241
	10	10	25	101

¹As millilitres of 0.1 N sodium hypochlorite per 100 gm. of fat.

Although the procedure outlined here is not strictly comparable to that described by Strohecker *et al.*, (31), there is sufficient similarity to indicate that the use of sodium hypochlorite is unsuitable for the determination of water-soluble, oxidizable compounds.

Physical Measurement of Hardness of Fat

Although a variety of physical methods have been proposed for determining the hardness of fat, it was considered desirable to investigate the possible utility of a machine designed for studies on the tenderness of meat because of its simplicity and ease of operation (37). Studies were made in which the machine was used either as a modified penetrometer, i.e., a definite weight was added to the lever arm and the time required for the jaws to pass through a definite distance determined, or in a manner similar to that employed for meat, i.e., a continuously increasing load was applied at a constant rate. These studies showed that the results obtained when a load was increased at a constant rate could be interpreted more readily, and gave more reliable information than those secured by the penetrometer type of measurement; considerable variation in hardness existed within any one sample of fat; and, since pork fat is not deposited generally as a homogeneous mass, but in at least two distinct layers corresponding to variations in feeding practice, the measurements could best be made by cutting across rather than down through the layers. However, considerable care was required to prevent the mechanical separation of the layers of fat when the force was applied at right angles to them. All measurements on hardness were made at 3° C.

Iodine Number

Of the variety of methods available, that of Kaufmann was selected and found to be entirely satisfactory (20). Moreover, in addition to giving results comparable to those by commonly accepted procedures, such as the Hanus or Wijs (9, 20), it has the advantage of ease of preparation of a solution that is free from obnoxious vapours, readily handled, and relatively stable. Moreover, if the titre should decrease, it can be readily adjusted to its original level by the addition of bromine.

Melting Point

Although the estimation of the melting point gives primarily an indication of fat quality, it is also indirectly related to rancidity. The measurements were made by means of the capillary tube method (1) in conjunction with a commercial, electrically operated melting point apparatus*.

Refractive Index

The ease and rapidity with which the refractive index can be determined has given prominence to the refractometric method of examining oils and fats. It is specifically related to fat quality, but is also affected by the development of rancidity. All readings were made with an Abbé refractometer at 40° C. (1).

Moisture and Volatile Content

Because of the varied opinions expressed as to the effect of moisture on the development of rancidity in fats (13, 16), it was considered desirable to include this measurement in the present investigation.

* A. Gallenkamp and Co. Ltd., London, England.

This was determined by heating finely chopped samples *in vacuo* at 25° C. for 16 hr., or to constant weight.

Total Fat

The determination of total fat was included primarily to ascertain whether any relation existed between the hardness of bacon fat, and the amount of non-fatty material present. After determining the total moisture and volatile content, the samples were transferred to extracted, dried, and weighed thimbles, and extracted with petroleum ether (b.p. 50° C.) in a set of Soxhlet extractors, especially selected for uniformity in the period required for filling and draining. After extracting for 24 hr., a period found to give complete removal of ether-soluble components, the thimbles were removed, dried in a rapidly moving current of air at 100° C. for 45 min., cooled, and weighed.

Interrelation and Application to Bacon Fat

MATERIAL AND PROCEDURE

Samples of fat were removed from the hams of each of two sides received from 22 Canadian packing factories after the sides had been stored at 1.1° C. for a period of 10 days, and again after smoking at 43° to 46° C. for 14 hr. (8). After removal the samples were placed in sealed glass containers and stored at -29° C. (-20° F.) for approximately six months in order to permit the formation of readily measurable quantities of those compounds associated with rancidity.

Since it was impossible to make all the measurements on the large number of samples available, properties considered to bear possible interrelation were grouped, and determined for the samples from certain factories. Accordingly, determinations for free fatty acid content, hardness, iodine number, melting point, refractive index, and moisture and fat contents were made on samples of both smoked and unsmoked fat from the same eight factories; Kreis, aldehyde, and oxidation-reduction potential on those from seven factories; and water-soluble components on those from three factories. In addition, the peroxide oxygen content, preselected as giving the most satisfactory indication of rancidity, was determined for all the samples in order that a comparable determination for rancidity might be available between all three groups.

RESULTS

The large amount of data available necessitated the use of statistical analysis for their interpretation. The relative importance of possible factors contributing to the observed variations in the properties studied here was assessed by analyses of variance. Such factors include experimental and sampling error, differences between sides from the same factory and over-all differences in the product from various Canadian factories. The degree of interrelation of the quantities studied within themselves, and with other known facts concerning factory practice and properties of the sides (4, 6, 7, 35) was determined by the calculation of simple coefficients of correlation.

Separate computations were made for both smoked and unsmoked bacon in order to assess the suitability of the various measurements for, and the effect of known factors on, both types, and at two levels of rancidity.

Mean Values and Analyses of Variance

From the mean values of the various measurements on rancidity given in Table III, it may be seen that bacon fat is markedly unstable, even at the low storage temperature of -29°C . (-20°F .) employed, and that the smoking process has marked antioxidant effect. The relative tendency for sides of bacon to go rancid was an inherent property of the side, as indicated by the significant differences between sides for the peroxide oxygen content, the Kreis and aldehyde values of both smoked and unsmoked fat. This is of some importance since it suggests that the observed differences in factory and curing practices (4, 6) have less effect on the development of rancidity than inherent properties of the hog.

TABLE III

MEAN VALUES AND ANALYSES OF VARIANCE FOR THE MEASUREMENTS ON RANCIDITY IN SMOKED AND UNSMOKED BACON FAT

Property	Type of bacon	Mean	Variance attributable to:			
			Between sides†		Between factories	
			D.f.	Mean square	D.f.	Mean square
Peroxide oxygen ¹	Unsmoked	32	22	107**	21	129
	Smoked	9.9	22	98.3**	21	95.9
Kreis value ²	Unsmoked	129	7	1854**	6	1808
	Smoked	50	7	3217**	6	3051
Aldehyde value ³	Unsmoked	152	7	6821**	6	13808
	Smoked	74	7	12850**	6	15980
Oxidation value ⁴	Unsmoked	101	3	11.1	2	18.3
	Smoked	102	3	16.2	2	82.8
Acid value of water-soluble components ⁵	Unsmoked	0.74	3	0.138**	2	0.226
	Smoked	0.59	3	0.141**	2	0.580
Free fatty acid ⁶	Unsmoked	0.75	8	0.018	7	0.268**
	Smoked	0.91	8	0.070**	7	0.628**
Oxidation-reduction potential ⁷	Unsmoked	428	7	1036	6	1235
	Smoked	387	7	2820	6	1642

** Indicates 1% level of significance.

† Significance assessed by comparison with variance due to the sampling and analytical error.

¹ As millilitres of 0.002 N sodium thiosulphate.

² In arbitrary units as defined in the text.

³ As parts (CO) per million of fat.

⁴ As millilitres of 0.1 N sodium hypochlorite per 100 gm. of fat.

⁵ As mg. potassium hydroxide per 100 gm. of fat.

⁶ As per cent oleic acid.

⁷ In millivolts.

All of the methods studied for estimating rancidity, except the oxidation value, appeared capable of showing a distinction between a slightly and a very rancid fat. The use of sodium hypochlorite, as outlined, appears to be unsatisfactory for the determination of water-soluble, oxidizable materials. While the mean oxidation-reduction potential of the unsmoked and more rancid fat was greater than that of the less rancid, smoked fat, the data obtained were very variable, and their dependability is uncertain. However, further study is being given to this property because of its apparent importance.

As previously indicated, significant differences between sides were observed for the acidity of the aqueous extract. Since a similar observation was noted for the peroxide oxygen, Kreis, and aldehyde tests, and as the values increased with increase in the degree of rancidity of the fat, there is some indication that this measurement is capable of giving an indication of the development of oxidative rancidity. It suffers, however, from lack of sensitivity.

While the observed differences in free fatty acid content between sides were significant for the smoked fat alone, those between factories reached statistical significance for both the smoked and unsmoked fat. The reason for this difference in behaviour of the acid content of smoked and unsmoked fat between sides is obscure. The differences between factories may be a

TABLE IV

MEAN VALUES AND ANALYSES OF VARIANCE FOR THE CHEMICAL AND PHYSICAL CHARACTERISTICS OF SMOKED AND UNSMOKED BACON FAT

Property	Type of bacon	Mean	Variance attributable to:			
			Between sides†		Between factories	
			D.f.	Mean square	D.f.	Mean square
Hardness ¹	Unsmoked	2.25	8	0.095	7	0.179
	Smoked	2.19	7	0.776*	7	0.733
Iodine number	Unsmoked	52.7	12	8.78**	11	25.5*
	Smoked	53.6	12	9.25**	11	23.5
Melting point, ° C.	Unsmoked	43.4	12	2.78**	11	15.6**
	Smoked	43.7	12	2.44**	11	19.7**
Refractive index at 40° C.	Unsmoked	1.4582	8	41.5**	7	210*
	Smoked	1.4578	8	93.0**	7	142
Moisture and volatile content, %	Unsmoked	11.4	8	4.14**	7	6.45
	Smoked	9.72	8	1.17**	7	4.26*
Fat content, %	Unsmoked	94.8	8	0.360**	7	1.63*

* Indicates 5% level of significance.

** Indicates 1% level of significance.

† Significance assessed by comparison with variance due to the sampling and analytical error.

¹ In arbitrary units of work.

reflection of variations in the number and type of lipolytic micro-organisms present in the fat from different factories. It is of interest to note that the mean free fatty acid content of smoked fat was slightly higher than the unsmoked, and that in both instances the values were sufficiently low to suggest that spoilage in bacon fat due to excessive free fatty acid formation is normally of little importance.

Mean values and analyses of variance for the physical and chemical characteristics of smoked and unsmoked bacon fat are shown in Table IV. A significant difference in the physical measurement of hardness was obtained between sides for the smoked fat alone. It is not known why the unsmoked sides did not behave similarly. Although convenient and rapid in operation, the precision of the method was poor, and it is not recommended for use as described.

Differences in the iodine number were due primarily to variations between sides. There was, however, some indication from the data for the unsmoked samples that variations in grading practice existed between factories. While the mean iodine value of the fat of low peroxide oxygen content was slightly greater than that of the high, the magnitude of the difference was small. This shows that the iodine number, as determined, is little affected by the development of rancidity, and consequently is unsuitable for following oxidation changes in fat.

With one exception significant differences in the melting point and refractive index of the fat were obtained both between sides and factories for smoked and unsmoked fat. As two further measurements of fat quality, they confirm the previous conclusions, namely, that variations in the hardness of Wiltshire

TABLE V

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN PEROXIDE OXYGEN, KREIS VALUE, AND OTHER MEASURED PROPERTIES OF SMOKED AND UNSMOKED BACON FAT

Quantities correlated	Unsmoked fat		Smoked fat	
	D.f.	<i>r</i>	D.f.	<i>r</i>
Peroxide oxygen content with:				
Kreis value	12	.93**	12	1.00**
Aldehyde value	12	.78**	12	.31
Acid value of aqueous extract	4	.72*	4	.67
Oxidation-reduction potential	11	.74**	12	.48
Iodine number	22	-.11	22	.31
Melting point	22	-.16	22	.15
Refractive index	14	.16	14	-.05
Moisture and volatile content	14	.17	14	-.20
Fat content	14	-.20	—	—
Kreis value with:				
Aldehyde value	12	.66**	12	.99**

* Indicates 5% level of significance.

** Indicates 1% level of significance.

bacon fat are due not only to differences between sides, but also to non-uniform selection and grading in the various factories. The development of rancidity caused little change in the melting point, and a slight increase in the refractive index. However, the changes were too small to permit either measurement to be used for the estimation of rancidity.

While the principal differences in the moisture and volatile content of the fat were between sides, there was also some indication that it was affected by factory and curing practice. On the average, the moisture and volatile content of the smoked fat was approximately 10% less than that of the unsmoked.

Significant differences between both sides and factories were noted for the petroleum-ether-soluble fraction of unsmoked fat. It is possible that such variations are reflected in the hardness of the fat, since it would seem reasonable to assume that the greater the proportion of structural or non-fatty material, the harder the fat. Approximately 95% of the original weight of the fat was soluble in petroleum ether.

Correlation of the Properties

Of the methods studied for the estimation of rancidity, the peroxide oxygen and Kreis tests were most closely related at both high and low levels of rancidity, i.e., unsmoked and smoked fat (Table V). This suggests that the formation of peroxides is closely accompanied by that of the parent compound of epihydrinaldehyde (29). The peroxide oxygen content of the unsmoked fat was also significantly and positively correlated with the aldehyde value, the acid value of the aqueous extract, and the oxidation-reduction potential as determined by one, but not the other, of the two electrodes employed. The lack of significant correlation with the aldehyde value at the lower peroxide oxygen levels is presumably due either to a relatively greater experimental error, or more probably to a differential formation of the two materials. A relatively poorer precision probably accounts for the lower coefficients obtained with the acid values of the aqueous extract and the oxidation-reduction potential of the less rancid fat. In contrast to these, it is of interest to note that the Kreis and aldehyde values were more closely related at low than at high levels of rancidity.

Of the procedures outlined for the estimation of rancidity, the peroxide oxygen, Kreis, and aldehyde tests are considered to be most suitable. The precision of the aldehyde determination is poor, but it possesses the advantage of determining a portion of the materials directly responsible for the rancid odour and flavour (29). The modified Kreis test is the most sensitive of the three, has excellent precision, but the values vary considerably with the concentration of fat. The peroxide oxygen test is believed to be most satisfactory, since it agrees closely with the Kreis test, is less affected by variations in the concentration of the fat, and because of the simplicity and general applicability of the experimental procedure.

None of the coefficients between peroxide oxygen and the physical and the chemical characteristics of the fat considered to be related to the development of rancidity reached the level of statistical significance (Table V). Although the implications suggested by the signs and magnitudes of the coefficients are of theoretical interest, they do not merit discussion here.

TABLE VI
SIMPLE COEFFICIENTS OF CORRELATION BETWEEN PHYSICAL AND CHEMICAL
PROPERTIES OF SMOKED AND UNSMOKED BACON FAT

Quantities correlated	Unsmoked fat		Smoked fat	
	D.f.	<i>r</i>	D.f.	<i>r</i>
Iodine number with:				
Melting point	22	-.34	22	-.15
Hardness	14	-.01	13	-.06
Refractive index	14	.79**	14	.58*
Total fat	14	-.09	—	—
Melting point with:				
Refractive index	14	-.24	14	-.11
Free fatty acid	14	.25	14	.04
Hardness	14	.01	13	-.02
Refractive index with:				
Hardness	14	-.03	13	-.07
Moisture and volatile content	14	-.47*	14	-.18
Fat content	14	.31	—	—
Free fatty acid	14	-.80**	14	-.59**
Moisture and volatile content with:				
Hardness	14	-.08	13	.04
Total fat	14	-.40	—	—
Free fatty acid	14	.46	14	.28
Total fat with:				
Hardness	14	-.02	—	—

* Indicates 5% level of significance.

** Indicates 1% level of significance.

The results of the computation of simple coefficients of correlation between the physical and chemical properties of the fat studied are shown in Table VI. An increase in the iodine number was associated with a decrease in the melting point and an increase in the refractive index. In the more rancid fats the melting point was positively related to the content of free fatty acid. The significant negative coefficient for refractive index and moisture content may be a reflection of the accelerating effect of moisture on the growth of micro-organisms elaborating lipases. The moisture and volatile content was related negatively to the amount of fat present, and positively to the free fatty acid content. The former observation suggests that moisture is present mainly in the non-fatty portion of the fat.

It is of considerable importance to note the significant, negative relations obtained between the refractive index and the free fatty acid content. This suggests that the use of the refractive index for the rapid estimation of the degree of unsaturation of a fat is suitable only if the samples contain comparable amounts of free fatty acid.

TABLE VII

SIMPLE COEFFICIENT OF CORRELATION BETWEEN THE PEROXIDE OXYGEN CONTENT OF SMOKED AND UNSMOKED BACON FAT AND CERTAIN FACTORY AND CURING PRACTICES EMPLOYED IN THE MANUFACTURE OF WILTSHIRE BACON

Quantities correlated	Unsmoked		Smoked	
	D.f.	<i>r</i>	D.f.	<i>r</i>
Peroxide oxygen content of the fat with:				
Number of days before cure	15	-.32	—	—
Number of days in cure	15	.40	—	—
Number of days from cure to second sampling of the meat	15	.13	—	—
Number of days from cure to after smoking	15	.08	—	—
Oxidation-reduction potential of the pump pickle	18	-.38	—	—
Oxidation-reduction potential of the cover pickle	18	-.13	—	—
Oxidation-reduction potential of the spent pickle	18	.31	—	—
pH of the pump pickle	16	-.11	—	—
pH of the cover pickle	18	.34	18	.23
pH of the spent pickle	20	.11	—	—
Sodium chloride in cover pickle	18	-.12	18	-.28
Sodium nitrate in cover pickle	18	.35	18	.22
Sodium nitrite in cover pickle	17	.14	17	-.04
Protein in spent pickle	18	.05	18	.04
Increase in protein content of the pickle during cure	18	.33	18	.05
Number of pump stitches	19	.12	19	-.01

Interrelation of Plant and Curing Practices and Properties of the Meat with Peroxide Oxygen Formation

Although simple correlations between the peroxide oxygen content of the fat and certain factory and curing practices did not attain statistical significance (Table VII), it is of interest to note that the magnitude varied inversely with the number of days from slaughter to the beginning of cure, and directly with the days in cure and with time from cure to the second sampling of the meat, when the samples of unsmoked fat were taken (8). Of the remaining factors studied, the following appeared to be of greatest importance: the oxidation-reduction potential of the pump and spent pickles, the pH of the cover pickle, the sodium chloride and nitrate content of the cover pickle, and the increase in the protein content of the cover pickle during cure.

Of a number of properties of the meat considered to bear possible relation to peroxide oxygen formation, only two appeared to be of importance (Table

VIII). The peroxide oxygen content of the fat was significantly and positively related to the oxidation-reduction potential of the meat, and positively but not significantly to the nitrite content of the meat.

TABLE VIII

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN THE PEROXIDE OXYGEN CONTENT OF SMOKED AND UNSMOKED FAT AND CERTAIN PROPERTIES OF WILTSHIRE BACON

Quantities correlated	Unsmoked		Smoked	
	D.f.	<i>r</i>	D.f.	
Peroxide oxygen content of the fat with:				
Oxidation-reduction potential of the meat—1st sampling (No. 2 electrode)	30	.35*	—	—
Oxidation-reduction potential of the meat—2nd sampling (No. 2 electrode)	42	— .13	—	—
pH of meat	42	.04	42	— .12
Moisture content of meat	42	— .01	—	—
Sodium chloride content of meat	42	.14	—	—
Sodium chloride content of meat adjacent to fat	42	.10	—	—
Sodium nitrate content of meat	42	— .08	—	—
Sodium nitrite content of meat	42	.27	—	—

* Indicates 5% level of significance.

Acknowledgment

The author wishes to acknowledge the able technical assistance of Mr. G. S. Harbottle, Laboratory Assistant, National Research Laboratories.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

OCTOBER, 1941

NUMBER 10

THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

I. FACTORS INFLUENCING THE INCEPTION OF DIAPAUSE¹

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Abstract

This first in a series of five papers includes a review of the literature on diapause and an outline of the life cycle of the European spruce sawfly in Canada, especially the developmental stages within the cocoon. In studies of factors influencing the inception of diapause, evidence has been secured from offspring of stock from one-generation and two-generation areas that there are genetic differences within the species with respect to the capacity for development without diapause. Environmental factors are capable of bringing on diapause, and such factors are obviously operative during the development of the last seasonal generation of "emergent" field populations. However, analysis of weather conditions and incidence of diapause in such field populations failed to indicate correlation between the degree of diapause and any one environmental factor.

Introduction

The European spruce sawfly, *Gilpinia polytoma* (Hartig)³, discovered in outbreak proportions in the Gaspé Peninsula, Que., in 1930, has since then extended its distribution range in North America to some 150,000 square miles including the Maritime Provinces and parts of Quebec and Ontario in Canada, and the neighbouring states of the United States. Serious mortality of spruce has resulted in the Gaspé Peninsula, and severe defoliation though only moderate tree mortality in other heavily infested areas. Without doubt the insect is the most serious menace to the spruce forests experienced until now in eastern North America.

The problem has been under continual study by the Division of Entomology of the Dominion Department of Agriculture since 1931, and more recently, by the United States Bureau of Entomology and Plant Quarantine, besides

¹ Manuscript received in original form March 29, 1941, and as revised, July 25, 1941. This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April, 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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³ The European spruce sawfly in North America, until recently designated *Diprion polytomum* (Hartig), has been shown by Reeks (59, and a forthcoming paper), Smith (67, 68) and Balch (9), to be distinct from Hartig's *polytomum*, though identical with another European species not previously distinguished from *polytomum*. The genus *Diprion* was revised by Benson (15), a new genus, *Gilpinia*, being proposed to include several species in the *polytomum* group. The name *Gilpinia polytoma* is used in the present paper because a valid name for the recently recognized species is as yet unpublished.

various state services. The principal features of the bionomics, distribution, and damage have been described in a series of papers by Balch and co-workers (5-9, 11, 12), Atwood (1), Brown (24-27), Brown and Fleming (28), de Gryse and Brown (42), and Gobeil (40, 41), based on work in Canada, and by MacAloney (48, 49), Plumb (56), Baldwin (13), Dowden (36), McIntyre (50), and Peirson and Nash (54), based on work in the United States.

Diapause in *Gilpinia polytoma* has received brief mention in several of the published accounts. Investigations on this phase of the bionomics from 1932 to 1940 leave no doubt that diapause is of fundamental importance in the epidemiology of this insect in North America. Through variations in diapause the species is adapted to climatically different regions where a different number of seasonal generations is produced, and seasonal and regional variations in the degree of diapause are important in determining the nature of the infestations.

This series of five papers describes the results of: (1) studies of genetic and environmental factors in relation to inception of diapause; (2) laboratory and field studies of factors influencing the breaking of diapause; (3) analyses of intracocoon development in different climatic areas, and other bioclimatic relations; (4) studies of the influence of food and diapause upon reproductive capacity; and (5) studies of the role of diapause in epidemiology. There has been no opportunity to carry out purely physiological studies of diapause in the spruce sawfly.

Review of Literature on Diapause

The term "diapause", coined by Wheeler (82) to designate a temporary immobility between the dorsal migration of the embryo and its return to the ventral face of the egg, and extended by Henneguy (43) to include resting periods during any stage in the life cycle, is by Shelford (64) considered to be synonymous with true dormancy, and is defined as "—— a condition in which no further activity or progress can be induced until certain physiological changes of a physico-chemical character have taken place——". The term does not imply any specific causation of the arrest of development, but by definition excludes resting conditions temporarily brought about by subthreshold temperature, etc.

The literature on diapause in insects has increased to such proportions that it is impossible to attempt a complete summary here. Reviews by Shelford (64), Chapman (31), Uvarov (80), Cousin (32), Richards (61), Bodenheimer (16), and Wigglesworth (84) summarize the more important conclusions. The present review is necessarily restricted to brief reference to theories of causation and to the role of various factors associated with the phenomenon.

Sajo in 1896 (cf. 32, p. 301) considered the aestivation of *Entomoscelis adonidis* Palles to be due to narcotization brought on by the accumulation of toxic substances, a view similar to that of Baumberger (14), that diapause is attributable to the obstructive influence of inactive substances accumulated in the cytoplasm after excessive or innutritive feeding, and to Roubaud's (62 and

many later papers) hypothesis of auto-intoxication. The latter, in addition to explaining the diapause of the individual, envisages also the accumulation, from one actively developing generation to another, of an "hereditary patrimony of intoxication" which eventually causes the intervention of a diapause period of purification. Bodine (19) proposes a mechanism of diapause in which developmental behaviour is the result of a competition between two opposing factors or groups of factors, diapause intervening when the diapause factor reaches a quantity or potency above a threshold value, development commencing when the diapause factor is no longer able to suppress the developmental factor. Under these various interpretations, the destruction or reduction below a threshold value of the accumulated substances is conceived to be the essential process leading to the resumption of development. According to the hypothesis of Wigglesworth (83), diapause is considered to be due to the temporary failure of the growth promoting factor or hormone (cf. also 61, 84) though the failure might in turn be governed by a diapause factor.

Roubaud's hypothesis has been criticized from several angles. Parker and Thompson (53) failed to find distinct differences in the Malpighian tubules and fat body of diapause and non-diapause larvae of the corn borer. Fink (38) found the assumption of an excretory function of the fat cells to be unjustified, the urates and other products in the cells necessarily signifying nothing more than active metabolism within the fat cells. This, however, is a criticism of detail rather than of principle. Dawson (33) questions the assumption that a somatic acquirement (surcharge of excretory products) can pass on an increasing physiological handicap from generation to generation. Cousin (32), working with several muscid flies upon which the hypothesis was based, failed to find any evidence that diapause in these species is either obligatory, rhythmic, or inherent; on the contrary, it is entirely the result of suboptimal conditions during the life cycle of the individual affected by diapause.

The time at which diapause intervenes in the life cycle of a species is as a rule rigidly fixed. An outstanding example is the *Melanoplus* egg, in which diapause occurs at a definite morphological stage unless forestalled by appropriate treatment (66). A few exceptions include *Reduvius personatus* Linn. which may go into diapause in successive or alternate nymphal instars (58), *Popillia japonica* Newm., which may go into diapause in any one of the three larval instars, depending on the food and temperature (47), and the locust, *Acrydium*, in which nymphs in a growth diapause may occur simultaneously with adults in reproductive diapause (63).

The physiological state (accumulation of toxins, increase of the diapause factor, or inhibition of the growth factor) leading to diapause may be entirely under environmental control, may be genetically determined, or again may vary according to interaction of environmental and hereditary factors, and it is obvious that no one explanation will apply to all species, nor necessarily to different members of the same species. A few examples will indicate the possible variations.

Considering results with *Lucilia sericata* Meig., other Diptera, and the chalcid *Mormoniella vitripennis* Walk., Cousin (32) believes diapause to be the result solely of some unfavourable environmental factor, in the absence of which continuous development is secured. This view, propounded for species with constantly available food, short life cycle, and several to many overlapping generations, is qualified for naturally univoltine species. While on the whole discrediting the role of heredity in diapause, Cousin concedes that it would be extraordinary if univoltine species, accustomed to a fixed climatic rhythm, should yield at once to constant optimal conditions, though she expects that they would do so within a few generations.

Ditman *et al.* (34) concluded that the pupal diapause of *Heliothis armigera* Hbn. is due solely to low temperature during the period of larval development. Diapause in the larvae of *Loxostege sticticalis* (Linn.) is associated with unsatisfactory nutrition and low temperature during development (73), whereas reduced moisture content of the environment and of the host also lead to diapause (74).

Squire (70-72) shows the larval diapause in *Platyedra gossypiella* Saund. to be closely related to changes in the host, and only incidentally to climatic changes. Squire suggests that the remote causes of diapause resolve into a question of unfavourable free water balance, and that the ability of *P. gossypiella* to go into diapause is the result of an evolutionary process enabling the insect to maintain itself on cultivated species of cotton, which, unlike the wild perennial flowering species, have extended periods of dormancy.

The environmental factors leading to diapause may, however, be very obscure. Dawson (33) concluded that the pupal diapause in *Telea polyphemus* (Cramer) is not intrinsically related to nutrition, nor to the rate of larval development as governed either by feeding conditions or by temperature, nor to temperature conditions during the pupal period itself; the important factor appears to be the thermal decline during fifth instar development. This hypothesis, satisfactory for the naturally univoltine stock of Minnesota, is untenable for the bivoltine stock of Nebraska, indicating that the stocks are genetically dissimilar in their reactions to ecological factors.

The phenomenon of widely separated cause and effect may lead to confusion in distinguishing between diapause under control of environment and heredity. For example, incubation of bivoltine eggs of *Bombyx mori* Linn. at 80° F. or higher causes eggs of the subsequent generation to be univoltine, whereas incubation at 65° F. or lower causes no change in the voltinism (77, 79). Dawson (33) produced evidence that incubation of polyphemus eggs at 86° F. either induces pupal dormancy or increases sensitivity of the last larval instar to declining temperature, with the same ultimate effect. Similarly, exposure of very young *Loxostege* larvae to low temperature induces diapause in the full grown larvae even when maturity is realized at 90° F., diapause being entirely absent in larvae reared continuously at this high temperature (73).

In spite of this feature of delayed action of an environmental factor, there are instances where diapause cannot be attributed to environment. The

studies of Theodor (75) on *Phlebotomus papatasi* Scopoli are of particular interest because of the borderline between inherent and environmental factors. The fourth instar larvae in field populations go into diapause at the onset of winter rains and at a temperature drop to about 60° F. In insects reared in the laboratory under controlled conditions of temperature, moisture, and food, some of the larvae in occasional broods go into diapause for no apparent environmental cause, although it is known that a lack of, or changed composition of the food, over or under population, and lower temperature are all capable of bringing on diapause. The following facts, however, cannot be explained on the basis of environment: (1) the recurrence of small proportions of diapause larvae in most actively developing broods; (2) the gradual rise in numbers of diapause larvae, up to 90%, in the fall and first winter generation in uniform optimal conditions; (3) the appreciably increased duration of development at this same time; (4) the greater resistance of diapause larvae at this time to efforts to reactivate development; and (5) the return to normal brood development during late winter and spring. To Theodor, these results suggest the existence of a latent inclination to diapause and the influence of persistent cyclical factors. Wigglesworth (84, p. 68) cites Gierke on a similar lengthening of the duration of development of *Ephesia kuhniella* at constant temperatures during the winter months.

The persistence of the rhythm of development in bivoltine stock of the Chinese oak silkworm, *Antheraea pernyi* Guér., transferred from southern Crimea to the less favourable climate of the central area of the Russian Union, is reported by Karlash (44) and Zolotarev (85). Second generation larvae arising from broods started in May were killed by unfavourable conditions in the autumn; but when seasonal development was artificially retarded by cold storage of overwintering pupae until mid-June, first generation pupae were formed in the cool weather of early autumn and went into diapause exactly similar to that of second generation pupae in Crimea. Owing to the failure of climatic impresses during normal first generation development in the central area of Russia to overcome the inherent emergent tendency of the stock, it appears that the culture of the species in this area would depend upon artificial manipulation of the time of seasonal development.

The full grown larvae of the European corn borer may proceed with pupal development at once, or they may go into diapause for several months. Although Kozhanchikov (46) reports that diapause in Russian stocks of this insect is apparently due entirely to temperature conditions during larval development (since there is no evidence of hereditary differences in developmental behaviour of stocks from one-generation and two-generation areas in Russia) there is good evidence that these findings do not apply to American stocks of the insect. Babcock (2) shows that diapause is not exclusively due to environmental factors, since univoltine and bivoltine American stocks tend to persist in their respective types of development when transferred to areas occupied by stocks of the opposite type. Also, diapause (second generation) larvae of bivoltine stock respond with low mortality to develop-

mental conditions after a rest period lasting to November, whereas diapause larvae of the univoltine stock respond equally well only after a rest period lasting until March. Vance (81) reports additional evidence of physiologically distinct strains of the insect in America.

Babcock (3, 4) found that unusually dry conditions during diapause delayed spring emergence and reduced the proportion of bivoltine stock that developed beyond the first generation. The trend towards univoltinism was still greater after a second winter of dry conditions, leading Babcock to conclude that "once the seasonal rhythm has been changed by the continued impress of a certain type of climate, and has been maintained for generations, the reversal to the original type will be much more difficult than at the beginning of the process". The data, which pertain to the inception of univoltinism in a bivoltine stock, and not the converse, seem to the writer insufficient to warrant this conclusion. In fact, the delayed spring emergence may have retarded subsequent development to a time at which seasonal changes, per se, induced diapause, quite independently of any alleged physiological reconstruction of the organism as a result of its previous hibernation experience. The increase in the incidence of diapause in many species with advance of the season (21, 64, pp. 156-157, 70-72) presupposes no dependence on effects produced in a previous generation.

Diapause is more particularly under control of genetic factors in the silkworm, *Bombyx mori*, univoltine races of which have a prolonged embryonic diapause in each generation; other races produce one or more generations completely free of diapause. In crosses, univoltinism tends to be dominant (30, pp. 210-216) but segregation is not entirely clear-cut because of the influence of the somatic cells upon the developing germ cells, the resulting eggs having characteristics of the mother's race (77, 79). A similar result is obtained when ovaries are grafted into female larvae of a different racial stock (cf. 84, p. 9).

Different hereditary types of pupal development in *Deilephila euphorbiae* Linn., with respect to the pupal period (absence of diapause, or diapause of variable duration), respiratory rate, etc., have been described by Heller (cf. 16, pp. 149-152, 84, p. 68). Goldschmidt reports racial differences in *Lymantria dispar* Linn., where the races are univoltine but genetically distinct in duration of diapause in the egg and in the rate of larval development (cf. 16, pp. 149-152, 35, pp. 156-157).

The possibility of obscuring racial differences with respect to diapause, by crossing and production of heterozygotes, and the domination of genetic factors by prevailing environmental conditions, must not be overlooked. The bimodal distribution, including earlier (emergent) and later (diapause) progeny of single polyphemus moths reared in the laboratory, suggests to Dawson (33) that the Minnesota stock is heterozygous for voltinism. In nature, climate prevents the development of the emergent elements beyond a single generation and thus ensures their survival. In the laboratory the emergent elements continue development without diapause.

Characteristic physiological alterations during diapause include cessation of mitosis (65), and a reduced respiratory metabolism (18, 21, 69). Reduced metabolism is to be regarded as a result of diapause (20) rather than its cause as was suggested by Knoche (45) and Tuleschkov (78). Many other physiological characteristics during diapause have been discussed in recent literature, but are outside the scope of this paper.

The resumption of development of insects in diapause is promoted by a number of factors. The need for rest at low temperature is almost a universal requirement. The temperature may have to be near the freezing point (29), or merely below the threshold of development (2, 39). While diapause is most efficaciously overcome at low temperature, in some instances it may also be overcome gradually at developmental temperature (21, 66). The response to favourable temperature is often more rapid in relation to the duration of exposure at low temperature (17, 21).

The coincidence of resumption of development with moisture changes in the natural environment (23, 57) and the stimulating effect of water addition either during the normal diapause period (2, 3, 4) or at the time of its normal cessation (60, 71, 76) clearly indicate the important role of water in breaking diapause. High humidity is not as effective as contact water in most insect species.

Other stimuli appearing to alter the normal physiological processes and frequently leading to a breaking of diapause, include singeing, friction, wounding, electrical or mechanical shocks, irradiation, treatments with chemicals, etc. (22, 43, 55, 61, 63, 84).

Although the physiology of diapause is far from being understood, the adaptive value of the phenomenon is recognized as leading to a synchronization of the organism with its environment, especially where survival depends upon timely occurrence of a resistant stage. The latter is characteristically the stage affected by diapause (16, pp. 149-152, 84, p. 69). What appears to be an interesting exception occurs in the fall cankerworm, *Alsophila pometaria* Harris, pupae of which, formed in early summer, have a diapause lasting until autumn. Adults emerge and oviposit in November and early December. A considerable proportion of the eggs will hatch without exposure to subthreshold temperature (10, 39), and although the egg is very resistant to low temperature, the winter hardiness of the pupa is apparently unproved. The summer pupal diapause ensures that eggs occur at a season when their untimely hatch cannot take place.

Diapause in Relation to the Life Cycle

The spruce sawfly overwinters as a larva within a cocoon in the forest floor. Seasonal development commences in April to June, according to climate, and adults emerge after several weeks to lay the eggs singly in spruce needles. There are five feeding larval instars, and at the fifth moult there appears a non-feeding instar that evacuates the alimentary tract through the anus, drops to

the ground, and spins the cocoon. The cocooned larvae, in areas where there is a single annual generation, go into diapause that lasts until the following spring or for several years. In areas in which there are two or more annual generations, the cocooned larvae of the last seasonal generation go into diapause until the following spring or later, whereas those of the earlier seasonal generation go into diapause or develop at once, according to circumstance.

DEVELOPMENTAL STAGES WITHIN THE COCOON

The last larval instar of *Gilpinia polytoma*, as of numerous other sawfly species, has a number of successive developmental phases frequently but unsatisfactorily included under the term "prepupa". This looseness of definition is confusing in studying insects in which the successive larval phases, though not separated by a moult, exhibit a variety of habits. The spruce sawfly larva appearing after the fifth moult, though non-feeding, is active on the trees for a day or two and reacts similarly to the earlier instars; for convenience, this phase has been called the free-living sixth instar. Beginning with evacuation of the alimentary tract a day or two after the fifth moult, the reactions to light and gravity are reversed, and morphological changes associated with the later phases are initiated. The terms "eonymph" and "pronymph", used for sawflies by Eliescu (37), Nägeli (52), and others, and for *Exenterus abruptorius* (Thunberg) by Morris (51), are adopted here for the distinct phases of the cocooned prepupal larva.

The *eonymph*, or first larval phase within the cocoon (Fig. 1A), is practically filled with fat body in the form of a many-folded, single-layered blanket of large cells held together by connective strands. The ovaries, with ovarioles already quite distinct, measure about 0.55 by 0.25 mm. and lie within the folds of fat body, one on either side of the slender intestinal tract in the hind portion of the fifth abdominal segment. The rare males are identified by the cluster-like testes in the corresponding segment. The abdominal prolegs are withdrawn, and the wing rudiments appear through the integument of fixed material as small white lobes. The eonymphal phase of the prepupal larva is the phase in which diapause characteristically occurs.

The *pronymph* is the phase in which structural reorganization and development of imaginal rudiments begin. The body becomes shorter, thicker, nearly straight, the intersegmental constrictions more prominent, the thoracic segments swollen, and the abdominal prolegs further reduced (Fig. 1B). The earliest pupal structure to become apparent is the eye, which, beginning as a narrow arc under the posterior margin of the larval ocular sclerite (Fig. 1C) gradually enlarges and moves dorso-caudad, attaining its full size and red colour prior to the moult into the pupa (Fig. 1G). The ovaries of the fully developed pronymph are about 1.65 mm. long, with clearly defined oocytes and nurse cell follicles. The larval skin is loose and overlies a copious supply of moulting fluid. The duration of pronymphal development from the first evidence of the pupal eye to ecdysis averages about 17 days at a mean temperature of 49° F.

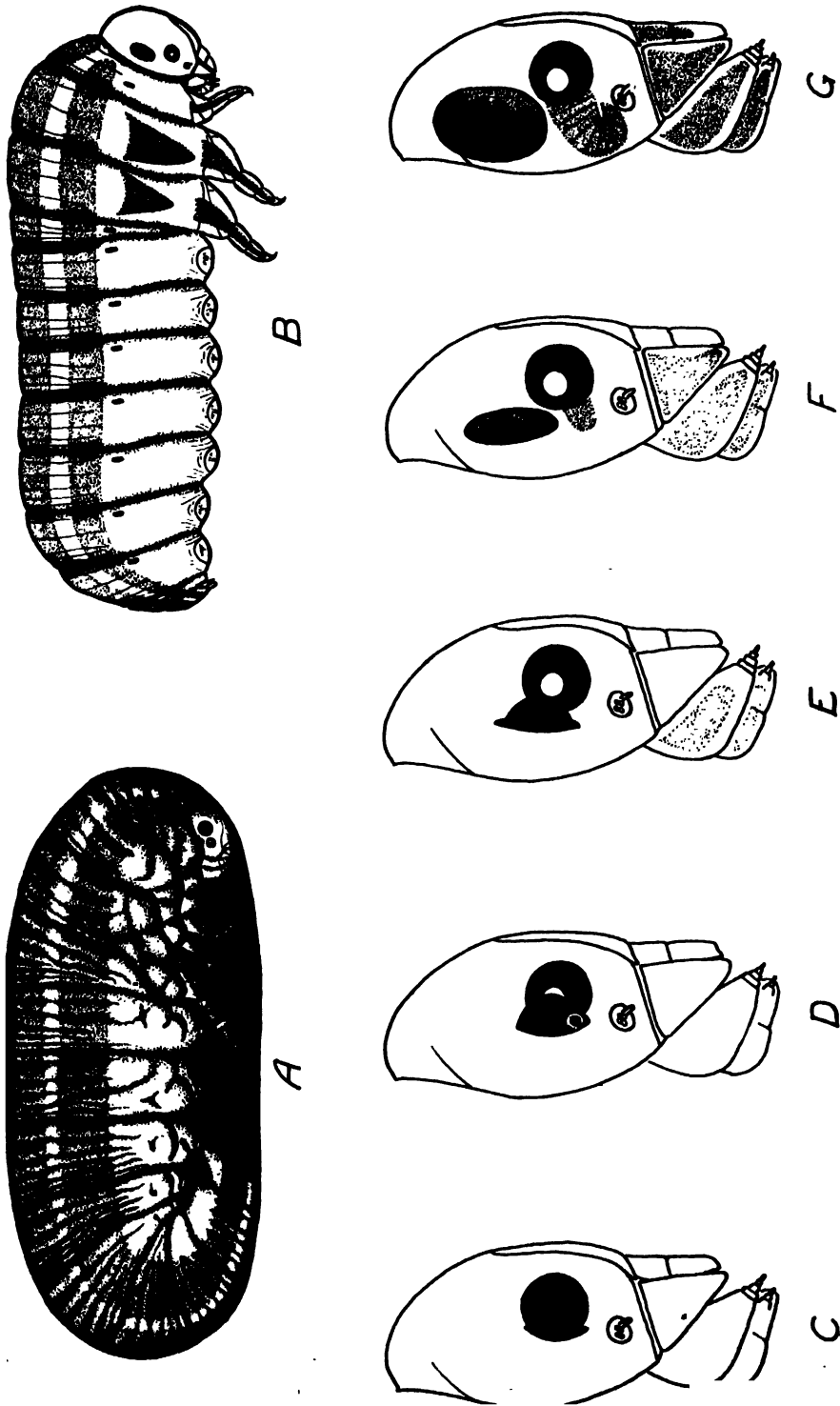


FIG. 1. Eonymph and pronymph of *Gilpinia polytoma*. A, eonymph within cocoon; B, advanced pronymph; C to G, head showing successive stages in pronymphal development. (Drawing of eonymph by W. A. Reeks.)

The first appearance of the pupal eye (Fig. 1C) has been used very extensively in these studies as a criterion of the continuance or resumption of development; its use in this respect is equivalent to that of the male gonads in European corn borer larvae by Parker and Thompson (53).

The pupal stadium averages 11 to 12 days at a mean temperature of 54° F., and 13 to 14 days at 50° F.

The newly transformed adult remains in the cocoon one to several days during which it dries and hardens. By thrusting the left mandible through the cocoon wall, then revolving inside the cocoon to shear off a lid, the adult escapes.

GENERAL CHARACTERISTICS OF DIAPAUSE

The development of eonymphs in diapause is resumed only after one or several periods of rest at low temperature. The rate of metabolism during diapause is greatly reduced, even at favourable temperature conditions, as demonstrated by preliminary tests of oxygen consumption, by the very slight reduction in dry weight over extended periods, and by the lack of a consistent downward trend in reproductive capacity of females emerging in successive years from a common source.

Pronymphs whose development is curtailed by the onset of winter conditions occasionally occur in a state of true diapause, resuming development only after a period of cold-rest. Pronymphal diapause was detected in overwintering Gaspé populations in 1934 and 1935, when from 2% to 8% of the overwintering individuals in different localities consisted of pronymphs in the early stages of development. Pronymphs in sample lots incubated prior to mid-November remained in diapause indefinitely, whereas there was a prompt resumption of development in sample lots incubated in mid-November and later. More typically, pronymphs occurring in the overwintering populations in Gaspé and in south central New Brunswick have continued their development irrespective of a rest period. Pronymphal diapause may therefore be regarded as unusual, and has never been observed during the summer season.

HARDINESS OF EONYMPHS IN DIAPAUSE

Eonymphs in diapause are very resistant to low temperature. The first mortality, about 1%, results from exposure of the cocoons to 0° F., and complete mortality results only after exposure to -20° F., when the exposure is gradual, such as experienced during a cold night in winter. Lethal temperatures are never experienced in the moss layer of the natural habitat (9).

Pronymphs are approximately as resistant to intense and prolonged cold as the eonymphs, but pupae and adults are much more susceptible. In a series of cocoon samples containing pupae and unemerged adults, that were held in storage close to 32° F. for several months and then opened for analysis, pupal mortality ranged from 35% to 67%, and adult mortality from 70% to 100%, in different sample lots.

Eonymphs are also more resistant to excessive moisture and to high temperature than are the later developmental stages within the cocoon. Many thousands of cocoons have been subjected to a variety of laboratory and field conditions in a study of the factors influencing resumption of development, providing extensive mortality statistics for each stage. It has generally been noted that the same environmental conditions that promote a high percentage of development also cause an increased mortality*. In the tabulation of representative mortality statistics (Table I), the death rate for each stage is the percentage relation between the number of dead individuals found at analysis of the cocoons, and the total number of individuals that attained, or developed beyond, the stage in question. The calculations therefore provide strictly comparable statistics for the various stages and for different experimental lots.

Mortality generally increased at higher temperature and in more frequent contact with water. Eonymphal mortality was generally the lowest and

TABLE I

MORTALITY STATISTICS FOR THE VARIOUS STAGES WITHIN THE COCOON IN RELATION TO TEMPERATURE AND MOISTURE CONDITIONS DURING THE PERIOD OF DEVELOPMENT

Material	Incubation conditions			No. of cocoons	Total devel., %	Mortality rate, %			
	Temp.	Rel. hum., %	Immersion			Eonymphs	Pro-nymphs	Pupae	Adults
Gaspé cocoons	74° F.	100	1 †	305	75.4	0.7	5.2	15.0	2.9
			3 †	290	69.7	1.7	4.5	11.3	2.4
			7 †	284	85.9	4.2	14.3	25.4	4.0
			14 †	295	87.5	6.4	19.8	33.3	4.7
			21 †	289	82.7	7.3	28.0	36.5	6.5
Gaspé cocoons	55° F.	100	None	144	16.0	1.4	0	0	5.0
			2-day immersion	702	24.1	0.4	1.8	10.1	3.8
	65° F.	100	None	141	19.1	0	3.7	0	0
			2-day immersion	593	28.5	0.5	0.6	3.3	4.1
	74° F.	100	None	445	97.1	2.0	6.7	19.1	9.6
			2-day immersion	970	97.0	2.5	10.2	19.1	6.8
English Settlement, N.B. cocoons									
a. Collected in fall	74° F.	100	None	9743	98.8	1.0	4.8	14.9	12.4
2-day immersion			9573	98.9	0.8	4.3	15.1	9.1	
b. Collected in May	74° F.	100	None	623	98.4	1.0	5.1	18.6	17.9
2-day immersion			627	99.5	0.3	2.9	21.3	17.7	
c. Collected in May, left in natural habitat.									
Protected from rainfall				2598	32.6	1.1	1.2	5.0	7.5
Exposed to rainfall				4675	88.7	1.8	3.1	13.1	18.1

† Immersions at rate of one daily.

*For this reason, experiments on the rupture of diapause must not cease with the recording of emerged adults since these represent only a part of the total number of individuals whose diapause was successfully overcome.

pupal mortality the highest, though the latter was occasionally exceeded by adult mortality. Under natural conditions in the forest, mortality within the cocoon (exclusive of predatism) is generally less than in the incubator, seldom exceeding 1% in eonymphs and pronymphs and 2% to 3% in pupae and unemerged adults, during the summer season. Excessive rainfall significantly increases the mortality, as may be appreciated from the following tabulation based on records in five localities in northern New Brunswick and Gaspé during the very wet season of 1939 (over 20 in. of rainfall in July and August).

Stage	Mortality rate, %	Stage	Mortality rate, %
Eonymph	2.4	Pupa	18.3
Pronymph	4.5	Adult	11.8

The much greater resistance of eonymphs assumes an increased significance in view of the prolonged diapause period frequently experienced in this stage; the normal period of pronymphal and pupal development is two to three weeks, and the newly transformed adults normally remain in the cocoons for only a few days.

Factors Influencing the Inception of Diapause

GENETIC FACTORS

Populations of sawfly lines were extensively reared at Fredericton from 1934 to 1938, to determine the range of variability in developmental behaviour within the species, and to check the possibility of relationship between origin of the stock and its type of development. A brief summary of the conclusions derived from this project has been given by Balch (9), but in view of the importance of the conclusions in relation to the interpretation of regional differences in populations, a more complete account of the results appears to be warranted.

The practice is to place newly emerged females, singly, in small globe cages enclosing a spruce twig in contact with water in the basal portion of the cage. The original twig accommodates the full complement of eggs and serves as food for the young larvae. Beginning about the second instar, the larvae are transferred to fresh foliage at intervals of a few days. At 74° F., cocoons are spun in the foliage or in moss provided for the purpose, beginning about the 28th day. In emergent lines, adults appear from about the 38th day to the 45th day, but in non-emergent or diapause lines development ceases at the eonymphal stage and, if the cocoons are left indefinitely at the incubator temperature, it is not resumed. The cocoons are left in the incubator for at least a month, and are then either opened for analysis or put into storage for several months, after which they are again incubated. The populations have been reared mainly at temperatures between 70° and 74° F. Humidity,

although not readily controlled because of the foliage and because of the difficulty of securing circulation within the tulle-covered globes, was generally about 80% or higher.

Offspring of Gaspé Stock

Eighty-five females of Gaspé origin, taken at random from field collections over a period of years, produced families of cocoons in the incubator. Based on the first generation, 70 families with a total progeny of 373 cocoons, were classed as diapause lines since no development beyond the eonymph occurred without a period of cold-rest. The other 15 families were classed as emergent lines, development being uninterrupted in 72% of the 118 cocoons produced.

Second generations were obtained for 10 of the lines classed as emergent in the first generation. In five of the lines there was some emergence from the second generation cocoons. Altogether, 25% of the 398 second generation cocoons of the so-called emergent lines of Gaspé origin continued development without diapause.

The third generation was obtained for three lines that were emergent in the two preceding generations, and for one line that was carried on only by storing cocoons of each preceding generation. Only 5 of the 270 third generation cocoons continued development without diapause, and these all pertained to a line emergent in each preceding generation.

The last-mentioned line was carried to the fourth generation in the incubator, but all of the 27 cocoons produced went into diapause. Repeated attempts to establish a continuously developing line of Gaspé stock were unsuccessful, owing to the complete intervention of diapause in the early generations.

In addition to the populations reared in the incubator, 100 unrelated females of Gaspé origin were reared outside at Fredericton, starting in late May or early June and were therefore concurrent with the first generation of the field population normally developing in the Fredericton district. All 716 cocoons in 92 families went into diapause, and in the other eight families there was a partial emergence, affecting 8 of the 50 cocoons produced. This represents an emergence of about 1% from first generation outside rearings of Gaspé stock in the Fredericton district.

Offspring of New Brunswick Stock

Thirty-five females from different parts of south central New Brunswick produced first generation progeny in the incubator. On the basis of the first generation cocoons, seven families (53 cocoons), in which there was no development beyond the eonymph, were classed as diapause lines. In the other 28 families (296 cocoons), there was an emergence without diapause of 81%, and, in over one-half the families, of 100%. Six other families, having 68% emergence without diapause, started in the insectary and finished in the incubator, were all emergent lines.

Successive generations of nine lines, classed as emergent in the first generation, were reared in the incubator at the rate of about nine generations a

year. Usually a few eonymphs of each generation went into diapause, and occasionally all the progeny of one female, but as a rule from two to five families were reared for each generation of each line and the succession of generations was unbroken. Owing to the pressure of other work, several of the lines were discontinued after 6 to 12 generations, but one line was carried to the 22nd and another to the 23rd generation. Finally the stocks were wiped out in 1938 by a virus disease introduced from the field and since then it has been impossible to rear larvae beyond the earlier instars. This circumstance has prevented the testing of new lines from field stock.

The varying percentages of insects in diapause in the different generations of a line remained unexplained, but since the variations were not parallel in the populations reared concurrently they were obviously not due to cyclical or seasonal factors. There was a sufficient variability in the degree of diapause in the different lines, as shown in the accompanying synopsis, to suggest the possibility of inherent differences between lines in the capacity for continuous development.

Line	No. of generations	No. of cocoons	Insects in diapause, %
1B	23	631	29
5	6	169	12
9	6	210	29
14	22	2306	8
21	10	319	11
22	12	495	4
24	12	669	0.6
25	12	882	5
26	9	432	4

Other evidence of inherent differences in the capacity for continuous development was provided by a number of New Brunswick lines which, having a high degree of diapause in the first generation, were reared for two or three further generations to determine whether there might subsequently be a significant departure. The data in the synopsis below indicate that the determination based on the first generation was close to that based on the entire line.

Line	Insects of first generation in diapause, %	Number of generations	Number of cocoons	Average number of insects in diapause, %
21F	27	3	81	49
32A	86	3*	25	96
32D	100	4**	55	89

*All second generation cocoons went into diapause.

**Second generation reared after storage of first generation cocoons. Fourth generation cocoons went into diapause, and development was resumed only after two periods of cold-rest.

The data presented in the foregoing sections indicate distinct differences between the Gaspé and south central New Brunswick stocks of *Gilpinia polytoma*. Comparatively large proportions of the New Brunswick population can be established as continuously developing lines in optimal conditions, though a small and variable percentage of the progeny goes into diapause. But there also exist lines that either fail to develop beyond the first generation, or in which the tendency towards diapause is so great that subsequent generations are reared with difficulty. The Gaspé population, on the other hand, appears to be composed predominantly of lines in which diapause intervenes after a single generation, and, to a lesser extent, of partially emergent lines.

Transfers of Stock to New Climatic Areas

Wild stock from south-central New Brunswick and stock from line No. 14 were transferred to the interior of the Gaspé peninsula and reared in the open with check populations of Gaspé stock. The results are summarized in Table II.

TABLE II

PERCENTAGE OF INSECTS IN DIAPAUSE IN POPULATIONS REARED FROM NEW BRUNSWICK AND GASPÉ STOCK IN CENTRAL GASPÉ

Year	Stock	No. of families	No. of cocoons	Insects in diapause, %
1935	Gaspé (wild)	4	74	100
	McNamee, N.B. (wild)	2	46	100
	Nashwaaksis, N.B. (wild)	3	44	100
	N.B. line No. 14	6	59	44
1936	Gaspé (wild)	5	97	100
	N.B. line No. 14	1	19	89
1939	Gaspé (wild)	—	—	100*
	Douglas Harbor, N.B. (wild)	3	27	4

*A strict check on rearing was not obtained in 1939, but there was no emergence from several hundred of the earliest cocoons of the Gaspé field population.

Gaspé stock reared in these three and in previous years, and newly spun cocoons of the field population of central Gaspé from 1932 to 1939, consistently failed to continue development under Gaspé climatic conditions. Two isolated exceptions occurred out of many thousands of newly spun cocoons that were under observation. Wild stock from McNamee and Nashwaaksis produced only a single generation in central Gaspé, but there was sufficient development in the first generation progeny of stock from Douglas Harbour and line No. 14 to preclude the possibility that the result was due to chance. Clearly the developmental behaviour of these latter stocks under Gaspé climatic conditions was influenced by their genetic constitution.

The second generation rearings of these New Brunswick stocks in Gaspé developed to partly grown larvae that were caught by the onset of winter conditions in early October. It is highly improbable that any part of a second generation could ever survive in central Gaspé.

As for the transfer of Gaspé stock to south central New Brunswick, it has already been noted that only 8% of the "lines" took advantage of the improved climatic conditions to continue development without diapause. This affected approximately 1% of the first generation progeny, under conditions at which a large percentage of New Brunswick stock develops without diapause.

ENVIRONMENTAL FACTORS

Studies of environmental factors in relation to the inception of diapause were made possible by the existence of lines capable of continuous development at optimal conditions. Branches of these lines were reared at variable temperature and on different types of foliage. Field studies were carried out in relation to the inception of diapause in populations in a two-generation area.

Effect of Foliage

The incidence of diapause in seven lines at different seasons of the year is summarized in Table III. The foliage on which these were reared included the needles of all years; white spruce was the characteristic host, though red spruce was used to some extent for line No. 14. The percentage of insects in diapause varied from season to season, but the fluctuations were not consistent in the various lines, therefore providing no evidence of a significant correlation between seasonal effects of the foliage as a whole, and diapause.

The preferred food of the larvae is the older foliage, and forcing them onto the new foliage, especially of black and red spruce, results in slower development, smaller size, and reduced survival. Later in the season the larvae

TABLE III

INCIDENCE OF DIAPAUSE IN SEVEN LINES REARED IN THE INCUBATOR AT DIFFERENT SEASONS

Season	1B		14		21		22		24		25		26	
	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %	No. of cocoons	No. in diap., %
Jan. - Feb.	140	2	315	9	98	1	74	0	177	0	157	0	198	1
Mar. - April	15	20	410	10	57	4	131	0	65	0	11	18	40	2
May - June	89	18	202	2	94	21	44	5	89	1	74	16	29	14
July - Aug.	137	34	380	2	18	50	121	1	28	11	37	0	10	30
Sept. - Oct.	143	58	413	13	44	5	98	15	69	0	68	0	29	7
Nov. - Dec.	107	31	586	7	8	25	27	0	241	0	535	6	122	0
Average for line		29		8		11		4		0.6		5		4

occasionally feed on the new foliage and in severely defoliated forests this may be the only alternative to starvation. Accordingly, it is of interest to determine whether there is any relation between the type of food and diapause. Branches of line No. 14 were reared on various types of foliage for several generations while concurrent generations of the main stem of the line were maintained on the "standard host", i.e., old and new foliage of white spruce. The results of the tests are shown in Table IV.

TABLE IV
INSECTS IN DIAPAUSE IN BRANCHES OF LINE NO. 14 REARED IN THE INCUBATOR ON
VARIOUS TYPES OF FOLIAGE, %

	No. of generations	No. of cocoons	No. in diapause, %
1. Red spruce (old and new)	5	148	1.3
Standard host	5	341	5.3
2. Black spruce (old and new)	5	92	8.7
Standard host	5	251	5.2
3. White spruce, very rank growth	8	262	5.3
Standard host	8	351	6.3
4. White spruce, old foliage (Oct.-Dec., 1935)	2	23	0.0
Standard host	2	217	2.3
5. White spruce, old foliage (July-Dec., 1936)	4	152	18.4
Standard host	4	292	4.1
6. White spruce, new foliage (Oct., 1935-Jan., 1936)	3	64	1.6
Standard host	3	242	2.1
7A. White spruce, new foliage (July-Sept., 1936)	2	54	72.2
Standard host	2	145	0.7
7B. White spruce, new foliage (Oct.-Nov., 1936)	1	76	43.4
Standard host	1	49	22.5
7C. White spruce, new foliage (Dec., 1936)	1	49	0.0
Standard host	1	98	0.0

NOTE:—The "standard host" consisted of white spruce twigs with old and new foliage.

There were obviously no differences in diapause, attributable to the type of foliage, in Tests 1, 2, 3, 4, 6, and 7C. In test No. 5, the significantly higher degree of diapause on the old foliage alone would suggest some causal relationship between the absence of new foliage and inception of diapause. This interpretation cannot be accepted, firstly, because the new foliage is only rarely eaten in the presence of abundant old foliage; and secondly, because the duplicate test, No. 4, failed to provide similar results. The increased diapause in test No. 5 must be attributed to unknown factors, chance alone being a very remote possibility (P less than 0.01).

The increased diapause in insects reared on the newly formed needles of white spruce suggests a definite relationship between seasoning of the new foliage and inception of diapause. Thus in the insects reared earliest on the new needles (test No. 7A) there was the highest percentage of diapause; this percentage gradually declined until, in insects reared in late fall and winter (Tests 6 and 7C) both on the current year's foliage and on the standard host, it was identical. The chemical changes associated with ageing of the new needles have not been investigated, and even though the experimental evidence may be taken to support an hypothesis that diapause in emergent stock is associated with changing nutritive conditions of the host, such an hypothesis fails to account for, first, the success of incubator rearings at all times of the year on freshly cut foliage; and second, the virtual insignificance of the less palatable new needles in the diet of most field populations that nevertheless experience a sharp mid-seasonal increase in diapause in south central New Brunswick.

Effect of Temperature

Twenty-four families descended from the same great-grandmother in line No. 14 were reared in the incubator until the larvae were in the second and third instars. Nine families were then transferred to an environment with fluctuating temperature (39° to 71°, mean 54° F.), six families were left in the incubator, and the remaining nine families were placed alternately in the incubator and the lower fluctuating environment until they had experienced 20 daily exposures of 16 hr. each to the fluctuating temperature. At the time of spinning, all cocoons were returned to the incubator. As shown in the accompanying synopsis, there was a clear relation between temperature at which they were reared and incidence of diapause.

Temperature conditions during late larval development	Number of cocoons	Insects in diapause, %
73° F.	88	0
Alternating between 73° and cooler environment	103	31
Cooler environment	135	72

Five families of line No. 14 were started in the field in central Gaspé in August, 1935. From then until October 11 the temperature was low, averaging only 45° F., and had dropped below the freezing point on 18 occasions, the absolute minimum being 20° F. On the latter date, the larvae that were mostly in the third instar, were transferred to the incubator to complete their development. Of 23 cocoons produced, only one went into diapause.

From these two experiments it may be concluded, (1) that fluctuating and very low temperature during the first three instars does not necessarily induce diapause in the eonymphs; and (2) that diapause may be brought into an emergent line by fluctuating and suboptimal temperature during the latter

part of larval development. This recalls the conclusion reached by Dawson (33) that the fifth instar of the polyphemus moth (Minnesota stock) is the critical stage at which sensitivity to temperature determines the course of subsequent development. Unfortunately, attempts to localize the suspected critical stage in *Gilpinia polytoma* were fruitless owing to the destruction by disease of about 100 family populations of pure line stock set up for a complete experiment.

The results described above were checked by rearing concurrently branches of pure lines in the incubator and in the open insectary at Fredericton (Table V). Diapause in the incubator insects was uniformly low, and while variable in the early outside populations (31% to 51% in 1935; 0% in 1936), it was 100% in all late outside populations. The following observations were of particular interest: (1) the 100% value in late outside populations applied equally as well to progeny of females reared in the incubator during the preceding generation, as to progeny of females reared outside in the preceding generation; (2) the diapause induced in the emergent lines was in most cases overcome by a short period of cold-rest, though without this, development was not resumed.

TABLE V

SUMMARY OF THE INCIDENCE OF DIAPAUSE IN THREE PURE LINES REARED OUTSIDE AND IN THE INCUBATOR AT FREDERICTON, N.B.

Line	Year	Time when rearings started	Outside		In incubator	
			No. of cocoons	No. in diapause, %	No. of cocoons	No. in diapause, %
5	1935	Early June	26	46	58	2
		Late July-early August	96	100	—	—
	1936	Early June	19	0	—	—
		Late July-early August	54	100	—	—
9	1935	Early June	13	31	27	11
		Late July-early August	59	100	—	—
14	1935	Early June	55	51	51	2
		Late July-early August	30	100	48	6
	1936	Early June	37	0	118	0
		Late July-early August	135	100	114	1

The temperature fluctuations during larval development in the outside populations of pure line stock at Fredericton and in Gaspé are represented graphically in Fig. 2. Due to the fact that not all larvae within each group developed simultaneously, it has been necessary to chart the fluctuations for the period during which the preponderant number of individuals in each group developed. A summary of the temperature conditions during each period, and during the final 13 days of each period (average fifth stadium), as well as the average percentage of insects in diapause, are shown in the insets

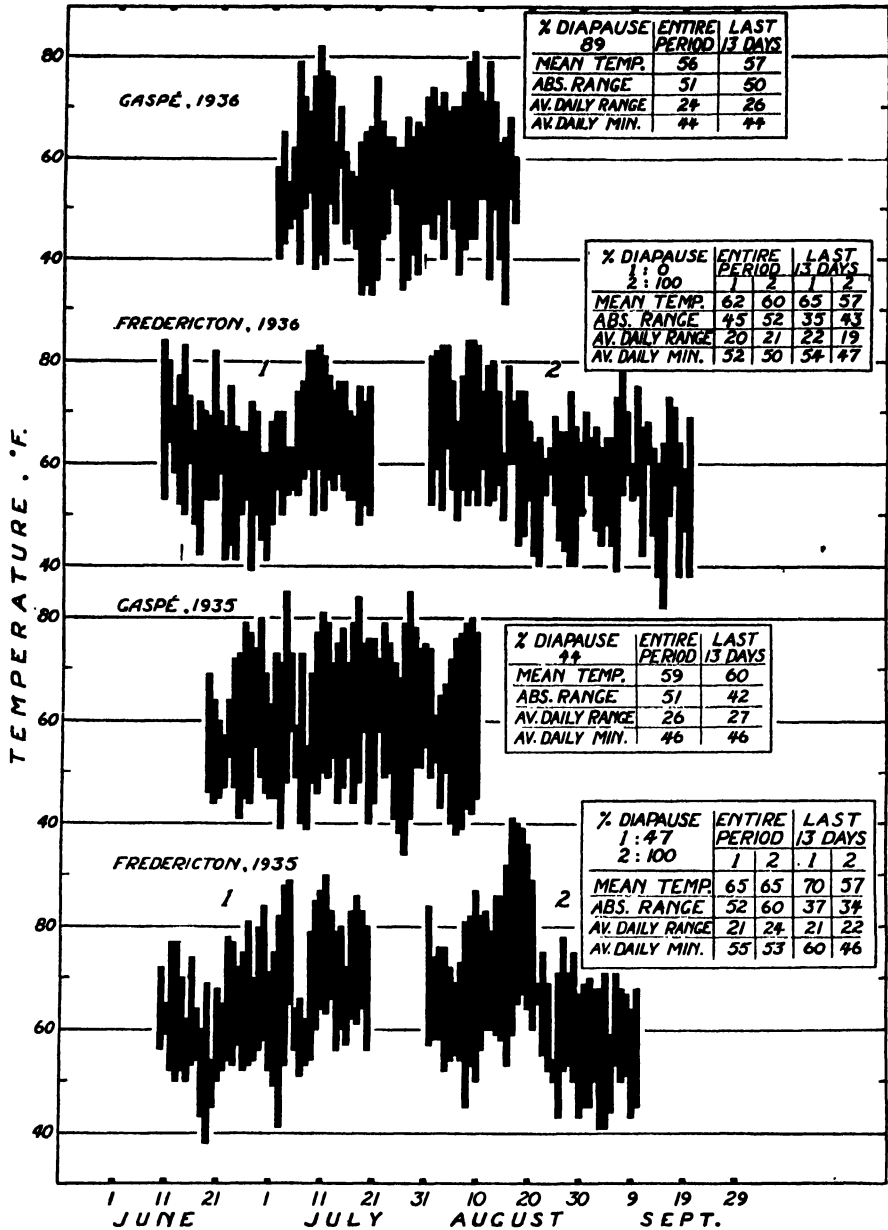


FIG. 2. Temperature fluctuations during outside rearings of emergent pure lines at Fredericton, N.B., and in central Gaspé, 1935 and 1936. Summary of temperature conditions and percentage of diapause in insets of the figure.

of the graph. There is no significant correlation between the mean temperatures during the entire period of first and second generation development at Fredericton, and the percentage of insects in diapause. The mean temperatures during the final 13 days of each period, which reflect the late seasonal drop in temperature in the Fredericton district, are more consistently related

to the degree of diapause. For a more critical analysis, however, it is instructive to compare temperature conditions during the rearing of the second generation populations at Fredericton with the temperature conditions for the single generation populations in Gaspé. For convenience, the pertinent statistics are shown in the following synopsis.

Year	Statistic	Insects reared in Gaspé	Second generation reared at Fredericton
1935	Basis: the entire period		
	Insects in diapause	44%	100%
	Mean temp.	59	65
	Av. min. temp.	46	53
1936	Insects in diapause	89%	100%
	Mean temp.	56	60
	Av. min. temp.	44	50
1935	Basis: the last 13 days		
	Insects in diapause	44%	100%
	Mean temp.	60	57
	Av. min. temp.	46	46
1936	Insects in diapause	89%	100%
	Mean temp.	57	57
	Av. min. temp.	44	47

Whether comparison be made on the basis of the entire developmental period, or on the basis of the last 13 days, it is evident that a considerable degree of development took place in the Gaspé rearings (July–August) at temperature conditions that were either approximately equal to or less favourable than those at which all the progeny of the Fredericton rearings (August–September) went into diapause. This feature is discussed further in the following section.

INCEPTION OF DIAPAUSE IN FIELD POPULATIONS

Fifth and sixth instar larvae were collected periodically in woodlands in south central New Brunswick and placed in outside cages supplied with foliage and moss. After a short interval the cocoons were removed to wire containers in the soil and subsequent emergence recorded daily. These studies were carried out in three localities for several years, with consistent results. The most complete series were obtained at Young's Brook and Kingsley Road, York County, and the data are summarized in Tables VI and VII. The series for each locality and year showed a progressive decrease in emergence, due to increased incidence of diapause, according to the lateness of larval maturity.

The results of the 1938 Kingsley Road series, with temperature and precipitation records, are shown graphically in Fig. 3. The completeness of the data warrants an analysis of the relationship between weather and the incep-

TABLE VI

SUMMARY OF SUMMER EMERGENCE FROM COCOONS SPUN AT DIFFERENT PERIODS, YOUNG'S
BROOK, YORK CO., N.B. (W. A. REEKS)

Lot No.	Time of spinning	No. of cocoons	Emergence, %	Mean date of emergence
1937				
1	June 28 - July 7	19	52.6	July 23
2	July 7 - 23	416	6.0	Aug. 9
3	July 14 - 27	509	6.5	Aug. 17
4	July 20 - Aug. 3	724	3.3	Aug. 16
5	July 23 - Aug. 5	836	3.3	Aug. 18
6	July 28 - Aug. 11	858	1.1	Aug. 21
7	Aug. 4 - 14	624	1.3	Aug. 25
8	Aug. 10 - 23	847	0.9	Sept. 10
9	Aug. 17 - 27	763	0.5	Sept. 15
10	Aug. 25 - Sept. 5	846	0.1	Sept. 28
11	Sept. 2 - 12	645	0.1	?
12	Sept. 8 - Oct. 1	621	0.0	—
13	Sept. 16 - Oct. 7	802	0.0	—
1938				
1	July 14 - 21	198	47.0	Aug. 5
2	July 20 - 28	376	38.6	Aug. 11
3	July 27 - Aug. 4	755	8.9	Aug. 17
4	Aug. 3 - 11	1041	2.6	Aug. 28
5	Aug. 10 - 18	918	0.5	Sept. 10
6	Aug. 17 - 26	757	0.5	?
7	Aug. 27 - Sept. 1	121	0.0	—
8	Sept. 2 - 9	34	0.0	—
9	Sept. 10 - 14	171	0.0	—
10	Sept. 15 - 26	614	0.2	?
11	Sept. 27 - Oct. 11	138	0.0	—
1939				
1	July 11 - 25	129	65.0	Aug. 12
2	July 25 - 31	1128	49.5	Aug. 16
3	Aug. 1 - 8	285	15.4	Aug. 24
4	Aug. 9 - 14	65	18.5	Sept. 1
5	Aug. 15 - 21	40	10.0	Sept. 13

tion of diapause. The analysis may relate to the total period of larval development for each lot, or only to the period of fifth instar development, on the assumption that the latter may be the critical stage in which subsequent developmental behaviour is determined. In either case the appropriate calendar period must be delimited. Since most of the cocoons were spun within a few days of the larval collection date, and since in extensive rearings in south central New Brunswick the total developmental period averaged 30 to 31 days, and that of the fifth instar 13 days, the corresponding periods for each field sample have been approximated as the month and the 13-day period ending with the larval collection date. The analysis has been carried out for alternate sample lots and the statistics appear in Tables VIII and IX.

TABLE VII

SUMMARY OF SUMMER EMERGENCE FROM COCOONS SPUN AT DIFFERENT PERIODS, KINGSLEY ROAD, YORK CO., N.B. (C. C. SMITH)

Lot No.	Time of spinning	No. of cocoons	Emergence, %	Mean date of emergence
1938				
1	June 30 - July 14	4	100	July 22
2	July 6 - 21	396	93.7	July 31
3	July 13 - 28	939	63.3	Aug. 4
4	July 20 - Aug. 4	1647	38.6	Aug. 13
5	July 27 - Aug. 11	1747	16.4	Aug. 20
6	Aug. 3 - 18	2935	5.5	Aug. 26
7	Aug. 10 - 25	1093	1.7	Sept. 25
8	Aug. 17 - Sept. 1	290	2.8	Oct. 16
9	Aug. 24 - Sept. 8	340	1.8	Oct. 18
10	Aug. 31 - Sept. 15	360	0.6	Oct. 16
11	Sept. 7 - 22	640	0.0	—
12	Sept. 14 - 29	416	0.0	—
13	Sept. 21 - Oct. 6	281	0.0	—
14	Sept. 28 - Oct. 13	296	0.3	?
15	Oct. 5 - 20	84	0.0	—
16	Oct. 13 - 27	183	0.0	—
1939				
1	July 4 - 20	90	97.8	July 28
2	July 13 - 27	58	55.1	Aug. 11
3	July 19 - Aug. 3	182	41.2	Aug. 11
4	July 27 - Aug. 9	31	32.3	Aug. 14

TABLE VIII

RAINFALL AND TEMPERATURE CONDITIONS DURING THE APPROXIMATE TOTAL DEVELOPMENTAL PERIOD OF SAMPLE LOTS IN THE 1938 KINGSLEY ROAD SERIES, WITH PERCENTAGE OF INSECTS IN DIAPAUSE

Lot No.	Approximate total developmental period	Rainfall, in.	Temperature conditions during period*				No. in diapause, %
			Average daily mean temp.	Days with mean temp. 60° or lower	Days with mean temp. 50° or lower	Days with minimum temp. 40° or lower	
1	June 1-30	3.83	64	6	10	0	0
3	June 13-July 13	6.09	64	8	10	0	37
5	June 27-July 27	4.47	66	7	6	0	84
7	July 10-Aug. 10	4.16	70	1	0	0	98
9	July 24-Aug. 24	4.29	70	1	0	0	98
11	Aug. 7-Sept. 7	3.52	63	10	10	3	100
13	Aug. 21-Sept. 21	5.06	59	20	19	7	100

* Temperature readings (degrees F.) of maximum and minimum thermometers in standard penthouse, twice daily.

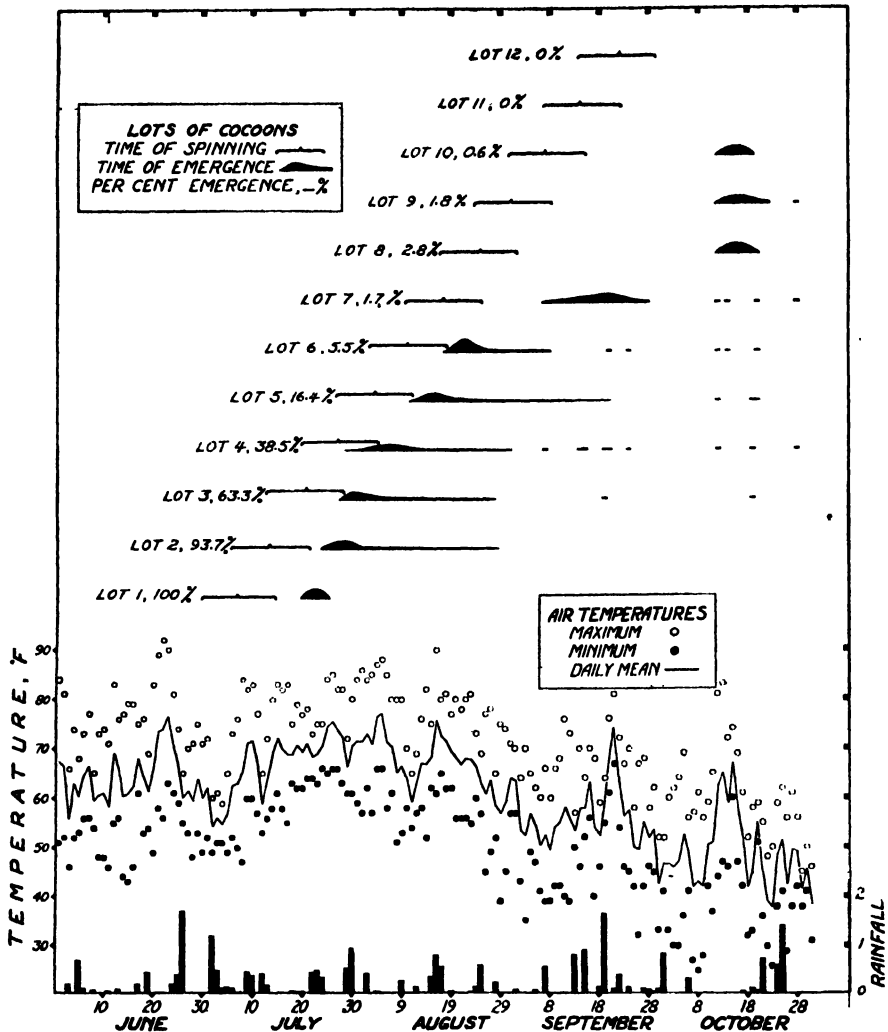


FIG. 3. Percentage and time of emergence from first generation cocoons spun at successive intervals, Kingsley Road, York County, N.B., 1938, correlated with temperature and precipitation.

Consideration of these statistics leads to the following conclusions: (1) Least diapause occurred in lots which developed at distinctly less favourable temperature conditions in June and early July, and the rapid increase in diapause was concomitant with a marked improvement in temperature during late July and early August. The slight increase in diapause (2%) in the latest samples coincided with late seasonal decline in temperature, but this has no practical significance in view of the trend established earlier. (2) There was no correlation between mean or minimal temperature within the range experienced, nor between precipitation in the various periods, and the degree

TABLE IX

RAINFALL AND TEMPERATURE CONDITIONS DURING THE APPROXIMATE 5TH INSTAR DEVELOPMENTAL PERIOD OF SAMPLE LOTS IN THE 1938 KINGSLEY ROAD SERIES, WITH PERCENTAGE OF INSECTS IN DIAPAUSE

Lot No.	Approximate 5th instar developmental period	Rainfall, in.	Temperature conditions during period				Insects in diapause, %
			Average daily mean temp.	Days with mean temp. 60° or lower	Days with mean temp. 50° or lower	Days with minimum temp. 40° or lower	
1	June 18-30	2.64	66	3	3	0	0
3	July 1-13	3.22	62	5	4	0	37
5	July 15-27	1.25	71	0	0	0	84
7	July 29-Aug. 10	1.72	70	0	0	0	98
9	Aug. 12-24	1.89	69	0	0	0	98
11	Aug. 26-Sept. 7	0.82	58	9	10	3	100
13	Sept. 9-21	3.44	59	10	8	3	100

of diapause. (3) Conditions after spinning of the cocoons were not obviously related to diapause, since the greatest increase in the latter occurred during late July and early August, although temperature conditions were equally favourable for about another month and precipitation rate was not significantly different at this period.

One further characteristic, viz., the variability of temperature conditions during larval development, remains to be explored. At this point it is pertinent to refer to Dawson's (33) experiments with Minnesota polyphemus larvae at controlled constant, and varying, temperatures; there was apparently no influence of low constant temperature upon the pupal diapause, but a gradually declining temperature during late larval development seemed to bring on diapause, even though the minimum temperature in the graded series was higher than the lowest temperature in the constant temperature series. Statistics on temperature variability during the fifth instar development of the spruce sawfly in the 1938 Kingsley Road series, are tabulated below.

Lot No.	Av. daily mean temp.	Absolute			Av. daily range	Insects in diapause, %
		Max.	Min.	Range		
1	66	92	48	44	22	0
3	62	83	47	36	18	37
5	71	85	55	30	16	84
7	70	88	51	37	23	98
9	69	90	52	38	20	98
11	58	78	35	43	23	100
13	59	81	39	42	20	100

The degree of variability in temperature was very great, both in the absolute scale and relative to the mean temperature, for Lots 1 to 3 in which diapause was least, and considerably less in the relative scale, though scarcely different in the absolute, for Lots 5 to 7 in which diapause was very high. It appears therefore that variability, per se, in temperature, can have little to do with the inception of diapause in the field populations.

Thus, although it is possible by laboratory experiments to show the influence of new foliage and low variable temperature on the inception of diapause, and although there are distinct differences in temperature conditions between early and late summer associated with absence or presence of diapause in emergent stock, an analysis of the data fails to provide evidence that the inception of diapause in the field population is intimately related to any one of the factors, food, temperature, or precipitation. The success of incubator populations throughout the year might also be taken to indicate the unimportance of sunlight and cyclical changes in the daylight period. It is, however, certain that diapause in emergent stock is determined environmentally, since the phenomenon is eliminated or relatively infrequent in the absence of environmental fluctuations. No one factor is likely to be the sole cause of diapause in *Gilpinia polytoma* and the only conclusion that can be reached is that the insect is evidently highly sensitive to a changing environment, with the consequence that diapause in emergent stock increases progressively with advance of the season.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

II. FACTORS INFLUENCING THE BREAKING OF DIAPAUSE¹

By M. L. PREHLE²

Abstract

Field and laboratory experiments have shown the importance of a period of "cold-rest" at a temperature below the threshold of development as a requirement for overcoming diapause in the spruce sawfly, especially in stock from a one-generation area. After cold-rest, maximal development results at a temperature of 74° to 75° F. or higher, and after contact with water. Temperatures in the field are lower and fail to promote so high development as may be obtained in the laboratory; however, temperature variations between 65° and 45° F. evidently have little influence on the degree of emergence from the diapause condition, though speed of development is directly affected. The benefit of contact with water is reduced or lost if contact occurs only while soil temperature remains below the threshold of development, and if the moisture taken up in the cocoon wall is lost by evaporation before it can be absorbed by the larva. The role of the cocoon in water exchanges, and differential effects of abnormal weather conditions upon intracocoon development in stocks in one-generation and two-generation areas, are described.

Approximately 200,000 cocoons have been used in laboratory and field studies of the factors influencing the breaking of diapause. The principal object of the experiments was to provide an understanding of the influence of factors operative in the natural habitat; the effect of artificial stimuli, such as chemicals, etc., has not been investigated. Space limitations require that the discussion be limited to the more important conclusions only, and that only representative tabulations of data be included.

Laboratory Studies

Overwintering cocoons collected in the autumn were divided into sample lots, some of which were opened for immediate analysis while the others were placed in various types of cold storage and periodically incubated under different temperature and moisture conditions. At 70° to 75° F., adults typically began to appear within two or three weeks and continued until the 8th or 10th week. At the conclusion of emergence, the sound cocoons were opened and the number of individuals that died when partially developed (i.e., beyond the eonymph) were added to the number of emerged adults in order to provide a measure of development.

Rest at Low Temperature ("Cold-rest")

In Gaspé cocoons incubated under favourable conditions of temperature and moisture in the early autumn there was only a low degree of development.

¹ Manuscript received in original form March 29, 1941, and as revised, July 25, 1941. This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April, 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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There was no general resumption of development over an indefinite period though many of the eonymphs were apparently healthy after six months or more in the incubator. Development gradually increased in samples left in cold storage for progressively longer periods, reaching a maximum for samples incubated in January or February. Typical results are shown in Table I.

TABLE I

DEVELOPMENT OF GASPÉ COCOONS IN RELATION TO THE DURATION OF COLD-REST

Cocoons were kept in storage in the ground until the dates indicated, then incubated under suitable conditions of temperature and moisture

1933-1934		1934-1935		1935-1936	
Date	Development, %	Date	Development, %	Date	Development, %
Nov. 8	16	Nov. 2	10.0	Nov. 1	2.7
Nov. 18	14	Nov. 16	11.8	Nov. 15	4.2
Dec. 2	21	Nov. 30	21.1	Nov. 29	29.0
Dec. 16	19	Dec. 14	28.8	Dec. 13	40.0
Jan. 2	50	Dec. 28	39.0	Jan. 3	70.8
Jan. 15	51	Jan. 11	30.7	Jan. 20	61.1
Jan. 29	61	Jan. 25	44.5	Jan. 30	65.5
Feb. 13	60	Feb. 8	40.8	Feb. 14	64.8
Feb. 27	71	Feb. 15	46.4	Feb. 22	72.9
Mar. 10	62	Feb. 22	56.0	Mar. 2	64.2
Mar. 24	65	Mar. 1	58.5	Mar. 10	68.5
April 4	64	Mar. 8	49.3	Mar. 18	61.3
April 19	71				

On the other hand, very high development resulted in all series of New Brunswick cocoons incubated at 74° F. and 100% relative humidity from early December onwards. Representative series are summarized in Table II.

TABLE II

DEVELOPMENT IN NEW BRUNSWICK COCOONS SHOWING LACK OF CORRELATION WITH EXTENDED COLD-REST

Cocoons were kept in storage in the ground and incubated on the dates indicated

1938-1939		1939-1940	
English Settlement, York Co.		Kingston, Kings Co.	
Date	Development, %	Date	Development, %
Dec. 13	99.4	Dec. 1	93.6
Dec. 27	99.2	Dec. 20	93.5
Jan. 10	96.5	Jan. 11	92.9
Jan. 24	96.8	Feb. 1	95.2
Feb. 7	99.6		
Feb. 21	99.3		
Mar. 7	98.2		
Mar. 21	98.7		
May 5	97.9		

Temperature during Cold-rest

The results of experiments in which Gaspé cocoons were incubated under favourable conditions of temperature and moisture, after extended cold-rest in various types of storage, are summarized in the following synopsis.

Year	Storage conditions	Development, %
1934-1935 (August collection)	a. In ground	46.6
	b. 30° to 45° F., av. 35° F.	45.2
	c. 35° alternating with 14° F.	46.0
	d. 14° F.	42.0
1934-1935 (September collection)	a. In ground	52.7
	b. 30° to 45° F., av. 35° F.	45.0
	c. 35° alternating with 14° F.	51.1
	d. 14° F.	22.1
1935-1936	a. In ground	66.1
	b. 33° to 52° F., av. 40° F. (moist sand)	62.8
	c. 33° to 52° F., av. 40° F. (79% R.H.*)	72.2

*Throughout the paper R.II. refers to the relative humidity.

Cold-rest at temperatures continuously above the freezing point was as effective as at fluctuating temperatures in the ground, mostly below the freezing point, or at widely variable temperatures (35° to 14° F.). Although development in the September series of 1934-1935 was uniformly low after storage at 14° F., the August series stored in the same location showed no significant reduction. The results failed to indicate any significant relation between the degree of cold, within these limits, and the resumption of development.

Other sample lots of the August and September collections were incubated at 50° F. in mid-October. There was virtually no emergence, and the samples were analysed after six months, giving the following results.

Collection made in:	Pronymphs, %	Pupae, %	Adults, %
August—			
Analysis in October	10.5	0	0
Analysis after six months at 50° F.	13.6	0	0
September—			
Analysis in October	8.6	0	0
Analysis after six months at 50° F.	8.1	0	0.6

The failure of the overwintering pronymphs to develop at 50° F. can be attributed only to the inadequacy of rest at this temperature for overcoming diapause, since the threshold of development is known to be below 45° F.

It may be inferred that cold-rest, to be effective, must be near or below the threshold of development, a condition that is always realized in the natural habitat during the winter months.

Moisture during Cold-rest

Results of an experiment to determine the effect of contact moisture during extended cold-rest upon subsequent development in the incubator are shown below.

Conditions of storage	Conditions of incubation	
	73° F., 70% R.H.	73° F., moist sand
	Development, %	
Mean temperature 40° F., 79% R.H.	4.8	72.2
Mean temperature 40° F., moist sand	10.2	62.8

With about 700 cocoons in each of the four experimental series, the difference between 4.8 and 10.2 is statistically significant, that between 62.8 and 72.2, marginal. Moisture during cold-rest therefore had a slight stimulating effect on subsequent development where no moisture was provided during incubation, but was without effect in cocoons that were kept moist during incubation. An adequate explanation of the distinction will be found in a later section.

Temperature during Incubation

The results of experiments involving over 5000 cocoons of Gaspé origin and dealing with the relation between incubation temperature and degree of development are summarized in Table III.

TABLE III

PERCENTAGE OF DEVELOPMENT IN RELATION TO INCUBATION TEMPERATURE

All lots had adequate cold-rest, were incubated at 100% relative humidity, and all factors except those noted were identical for the lots of each series

Treatment	Incubation temperature, °F.			
	45°	55°	65°	74 to 75°
	Development, %			
1938-1939				
1. No contact water provided	—	16.0	19.1	94.5
2. Immersed two days in water at 32° to 74° F.	—	24.1	28.6	96.9
1939-1940				
3. No contact water provided	63.7	55.7	71.0	98.8
4. Immersed two days in water at incubation temperature	74.3	58.7	73.0	99.1

The statistical significance of differences between the values may be briefly summed up as follows: Series 1,—16.0 and 19.1, not statistically different one from another, but both different from 94.5; Series 2,—relations as in Series 1; Series 3,—55.7 and 63.7, not different, 55.7 and 71.0, different, 63.7 and 71.0, not different, 98.8, significantly different from all other values; Series 4,—58.7 and 74.3, different, 58.7 and 73.0, different, 74.3 and 73.0, not different, 99.1, significantly different from all other values. Series 3 and 4 therefore provide, unexpectedly, some evidence that an incubation temperature of 55° F. is less efficacious as a stimulus to development than 45° or 65° F., though there is no clear evidence of a difference in the effects of incubation at 45° and 65° F. Series 1 and 2 partly counterbalance the first conclusion, since they fail to indicate significant differences between incubation temperatures of 55° and 65° F. On the whole, the evidence indicates that temperature differences within the limits of 45° and 65° F. have no notable effect upon the degree of development, but a temperature of 75° F. has a markedly more stimulative influence than one of 65° F. or lower.

Development at 45° was very slow, and the first pupae appeared after about seven weeks and the first adults after about 12 weeks, despite the fact that advanced pronymphs were present in the overwintering material at the time of incubation. The threshold of development is obviously only 2° to 3° below 45° F.

Moisture Conditions during Incubation

The importance of suitable moisture conditions as a factor in the resumption of development has been demonstrated for many insects, including the codling moth (5), ragweed borer (2) and the pink bollworm (3, 4), to mention but a few examples. Before describing the effect of moisture upon the resumption of development in the spruce sawfly, it is necessary to remark that divergent results were occasionally obtained when using experimental material of different origin, indicating that uncontrolled factors (physiological state of the overwintering populations) were quite variable. Typical results will be shown and attention drawn to others that were at variance.

Contact Moisture

Contact moisture was supplied by placing the cocoons on moist sand in covered dishes, by frequent momentary dipping, or by immersion for one to three days. Experimental results are summarized in Table IV; the incubator temperature was 74° to 75° F., and all cocoons were held at 100% relative humidity between or after treatments except where otherwise stated.

In general for Gaspé material, there was increased development as the duration of contact with water, or the frequency of dipping, was increased. Infrequent dippings (intervals of four to seven days) were of no benefit when the cocoons were in an unsaturated environment between dippings. Immersion for one to three days was equally effective in promoting development as contact of 14 to 21 days on moist sand, or 14 to 21 daily dippings. Mortality

TABLE IV

PERCENTAGE OF DEVELOPMENT AS INFLUENCED BY CONTACT WATER IN THE INCUBATOR

Year	Origin	Treatment	Development, %
1936-1937	Gaspé	0, check 1 day on moist sand 3 days on moist sand 7 days on moist sand 14 days on moist sand 21 days on moist sand	67.2 72.0 72.1 77.2 82.5 93.6
1937-1938	Gaspé	0, check 1 day on moist sand 3 days on moist sand 7 days on moist sand 14 days on moist sand 21 days on moist sand	20.3 60.6 83.4 80.4 92.4 88.0
1936-1937	Gaspé (cocoons held at 80% R.H. between and after dippings)	0, check 3 dippings, 7-day intervals 5 dippings, 4-day intervals 10 dippings, 2-day intervals 21 dippings, 1-day intervals	30.4 23.9 26.5 51.5 57.2
1936-1937	Gaspé	0, check 1 dipping 3 dippings, daily intervals 7 dippings, daily intervals 14 dippings, daily intervals 21 dippings, daily intervals	38.4 60.8 81.1 77.7 86.3 91.7
1936-1937	Gaspé	0, check 1-day immersion 2-day immersion 3-day immersion	38.4 77.1 80.4 80.9
1937-1938	Gaspé	0, check 1-day immersion 2-day immersion 3-day immersion	20.3 89.0 94.5 95.6
1938-1939	English Settlement, N.B.	0, check 2-day immersion	98.8 99.0
1938-1939	Douglas Harbour, N.B.	0, check 2-day immersion	96.5 98.2
1939-1940	Kingston, N.B.	0, check 2-day immersion	93.8 90.3

in the various developmental stages was frequently less after immersion for short periods than after the more prolonged contact with water.

Exceptional results were obtained in the 1938-1939 and in the 1939-1940 experiments with Gaspé material, equally high development resulting in cocoon samples incubated at 100% relative humidity, 74° to 75° F., whether or not contact water was supplied. It should also be noted that contact water at incubator temperatures of 45° to 65° F. has a reduced and doubtfully significant effect, indicating that the response to water addition is largely conditioned by incubation temperature.

In contrast with typical Gaspé material, all series of New Brunswick samples incubated at 74° F., 100% relative humidity, gave uniformly high development independent of contact water.

Temperature of Water during Immersion

Cocoons were immersed for two days in water at various temperatures from 32° to 86° F., and samples from each immersion temperature were then incubated at 100% relative humidity and at 55°, 65°, and 74° F. Development in the various samples, consisting of 100 to 150 cocoons each, is summarized below.

Immersion temperature, °F.	Incubation temperature, °F.		
	55°	65°	74°
	Development, %		
1. Check (not immersed)	16.0	19.1	94.5
2. 32°	24.7	27.9	96.4
3. 40°	25.7	27.4	96.4
4. 55°	21.8	—	96.5
5. 65°	20.6	25.5	97.2
6. 74°	27.7	33.6	97.8
7. 86°	97.2	98.5	87.1
Average, Series 2 to 6	24.1	28.6	96.9

The effect of the immersion temperature was partly obscured by the high development in all samples incubated at 74°. However, immersion at 86° greatly increased development in the samples incubated at 55° and 65°. In analysing the differences between the checks, and the combined values for all samples immersed at temperatures of 32° to 74°, for each of the incubation temperatures of 55° and 65°, the probability values are 0.07 and 0.05 respectively, indicating but not definitely proving that development was higher as a consequence of immersion, whereas the variations in the degree of development in samples immersed at temperatures between 32° and 74°, were for each incubation series at 55° and 65°, not greater than would occur as chance fluctuations, the probability in each case being approximately 0.75.

One may conclude from this experiment that the temperature of water during contact, within the limits of 32° and 74°, is of no importance, the essential requirement being the addition to the cocoon wall of water that is subsequently utilized by the contained larva if environmental conditions permit. These conditions include a temperature favourable to development and a humid external environment so that the moisture gained in contact is not lost by evaporation.

Atmospheric Humidity

Experiments were conducted to determine the influence of atmospheric humidity on degree of development when the cocoons were or were not given previous contact with water, and also when the contact with water was subsequent to various degrees of drying. In the experiments summarized in the following synopses, all samples had adequate cold-rest to ensure high development at suitable conditions, and all lots were incubated at 73° to 74°; contact with water was also at the same temperature.

a. Cocoons incubated without contact water.

1. Gaspé material, 1935-1936; overwintering pronymphs about 3% of the population.

R.H. in incubator, %	5-10	10	26	55	81	100	moist sand	Check,
Development, %	5.3	7.2	4.3	5.5	8.0	38.1	65.0	

2. Gaspé material, 1936-1937; overwintering pronymphs about 6% of the population.

R.H. in incubator, %	20	56	81	100	Check, moist sand 21 days,
Development, %	17.5	18.4	40.9	67.2	then 100% R.H. 93.6

3. Gaspé material, 1937-1938; overwintering pronymphs about 3% of the population.

R.H. in incubator, %	15	45	81	100	Check, 21 daily dippings,
Development, %	2.7	4.7	7.7	20.3	then 100% R.H. 82.7

b. Cocoons placed on moist sand for various periods, then transferred to different conditions of atmospheric humidity.

Gaspé material, 1936-1937; overwintering pronymphs about 6% of the population.

Initial period on moist sand	R.H. in the incubator, %			
	20	56	81	100
0, check	17.5	18.4	40.9	67.2
1 day	22.4	23.5	57.1	72.0
3 days	23.1	26.8	63.0	72.1
7 days	24.0	41.7	68.8	77.2
14 days	45.2	57.6	87.4	82.5
21 days	65.2	74.7	86.5	93.6

c. Cocoons held at 15% R.H., 74° F., for various periods, then transferred to 100% R.H. with or without immersion after the initial drying.

Gaspé material, 1937-1938; overwintering pronymphs about 3% of the population.

Period of initial drying (weeks)	Samples with no contact water at transfer	Samples immersed for two days at transfer
0, check	20.3	94.5
1	10.8	81.9
2	10.8	71.2
4	6.9	31.5
6	5.1	9.0
8	2.7	5.6

Bearing in mind that a small proportion of pronymphs was present in each experimental population, and that this must be considered in judging the influence of the various treatments, the experimental results warrant the following conclusions: (1) Development was inhibited in all unsaturated environments, but was not prevented altogether even in the driest environment; (2) the benefit of an initial contact with water for varying periods was reduced in relation to the degree of unsaturation of the environment to which cocoons were later exposed; (3) drying of the cocoons reduced the capacity for development when drying ceased, and also reduced the capacity to benefit from subsequent contact with water, in relation to the amount of initial drying, since there was practically no response after drying for eight weeks at 15% relative humidity, 74° F.

CRITICAL PERIOD

Rice (2) found that while emergence of *Epiblema strenuana* from its larval diapause was promoted by frequent wettings, the timing of the contact with water was extremely important. If the wettings were discontinued before the normal period of pupation, or were not started until several weeks afterwards, there was a marked reduction in the response to the same schedule of wettings. This indicated a critical period, corresponding to the normal pupation time in nature, at which the insect was most sensitive to moisture addition.

It has already been shown that the response of spruce sawfly material from Gaspé, to identical temperature and moisture conditions, becomes increasingly greater with cold-rest extending to January or February. The response at an incubator temperature of 74° is much greater than that which occurs in nature in the following summer. Repeated experiments, in which successive samples of cocoons were incubated under the same conditions at various periods during the late winter and the spring, have failed to provide any convincing evidence that the sensitivity of the insect to moisture addition varies in relation to the natural period of development. The difference in the degree of development realized in the incubator and in the field can therefore be attributed only to the differences in temperature and moisture conditions between the two environments.

INFLUENCE OF THE COCOON ON WATER EXCHANGES

The function of the cocoon in maintaining the micro-climate has been investigated by Ulyett (6) in studies of the physical ecology of *Microplectron fuscipennis* as a parasite of *Diprion sertifer*. From determinations of parasite development and survival at various saturation deficiencies of the external environment, and comparison of weight changes of naked and cocooned host larvae, Ulyett concluded that the micro-climate was kept near the saturation point by moisture from the host, and hence could not absorb moisture contained in the cocoon wall. The protective influence of the cocoon, broke down

only in very dry air, much more extreme than would normally be encountered in the natural habitat.

The problem of determining the role of the cocoon in regulating water exchanges in *Gilpinia polytoma* was of particular importance because of the relationship between moisture conditions of the external environment and the resumption of development.

Loss of Water

Studies of water loss in Gaspé cocoons containing a minimum (2 to 3%) of overwintering pronymphs, were carried out sufficiently early in the cold-rest period to avoid development within the cocoons during the progress of the experiments. The gross weight of 12 samples, each of 50 cleaned cocoons, was determined, and six samples were placed in desiccating jars at 75° F. The other six samples were dissected, the weight of the larvae and of the cocoon shells determined, then they too were placed in the desiccating jars. Weight determinations of the sound cocoons, naked larvae, and empty shells were made periodically to the 40th day, and then all larvae were brought to constant weight in the oven.

Data necessary for the interpretation of the experimental results are included in the synopses.

- a. Relation between gross weight and net larval weight at the start of the experiment
(All weights in centigrams)

Sample	Gross weight	Larval weight	Larval weight as percentage of gross weight
1	360.2	311.1	86.37
2	358.3	308.9	86.21
3	366.6	315.8	86.14
4	370.5	318.9	86.07
5	375.3	322.9	86.04
6	364.1	314.5	86.38
Totals	2195.0	1892.1	86.20

- b. Weight changes in naked larvae in 40 days

Average initial live weight	Saturation deficiency	Average weight at at end	Average oven-dry weight	Water content, %
6.22	22.0	1.98	1.97	68.3
6.18	16.5	1.98	1.95	68.4
6.32	12.8	2.04	1.97	68.8
6.38	8.4	2.12	1.98	69.0
6.46	4.2	2.37	2.03	68.6
6.29	0	—	—	—
Average of all lots: 6.31			1.98	68.6

c. Final weight of larvae left in the cocoons until the end of the experiment

Saturation deficiency	Average live weight at end*	Average oven-dry weight
22.0	4.55	1.93
16.5	4.92	1.98
12.8	4.98	1.92
8.4	5.41	1.98
4.2	5.69	1.96
0	6.19	1.99
		Average of all lots: 1.96

There was no mortality of larvae during the experiment.

The larvae constituted 86.2% of the initial gross weight, had an average live weight of 6.31 cg., and a water content of 68.6% of the live weight—assuming that weight loss during drying was entirely due to water loss. That this assumption is essentially correct is indicated by the fact that the average oven-dry weight (1.97 cg.) of the naked larvae at a saturation deficiency of 22.0 mm., where death supervened within a few days, was not significantly different from the oven-dry weight of naked larvae (1.95 to 2.03 cg.) in the more humid environments where death came gradually, or from the oven-dry weight of the cocooned larvae (1.96 cg.) which were all alive at the end of the experiment. It may be concluded that weight loss due to oxidation of reserve substances was negligible, and hence that the weight loss was virtually all due to loss of water.

The water loss from sound cocoons (Fig. 1, top panel) at each saturation deficiency was greatest during the first two days, and gradually approached direct proportionality to time. In the strict sense, however, the regression of water loss on time was slightly curvilinear over the entire 40-day period. The slight loss of water in the so-called saturated environment was apparently due to the periodic disturbance at the successive weighings. The ultimate oven-dry weight of the contained larvae (1.99 cg.) was exceeded by only one other sample, so the loss from the sound cocoons in so-called saturated environment cannot logically be attributed to loss of reserve substances through metabolism.

Naked larvae lost water rapidly, almost independently of saturation deficiency within the limits of 4.2 to 22.0 mm. (Fig. 1, middle panel), and the loss in 40 days in the driest environments was essentially the entire water content. In the dry environments, the first deaths occurred when water loss was about 40%, and all larvae were dead when the loss reached 60% of the initial live weight. There was also a considerable loss of water in the humid environment; the first mortality occurred when water loss was 10%, and all larvae were dead in 12 days with an average loss of 23.12%, and were discarded because of mold.

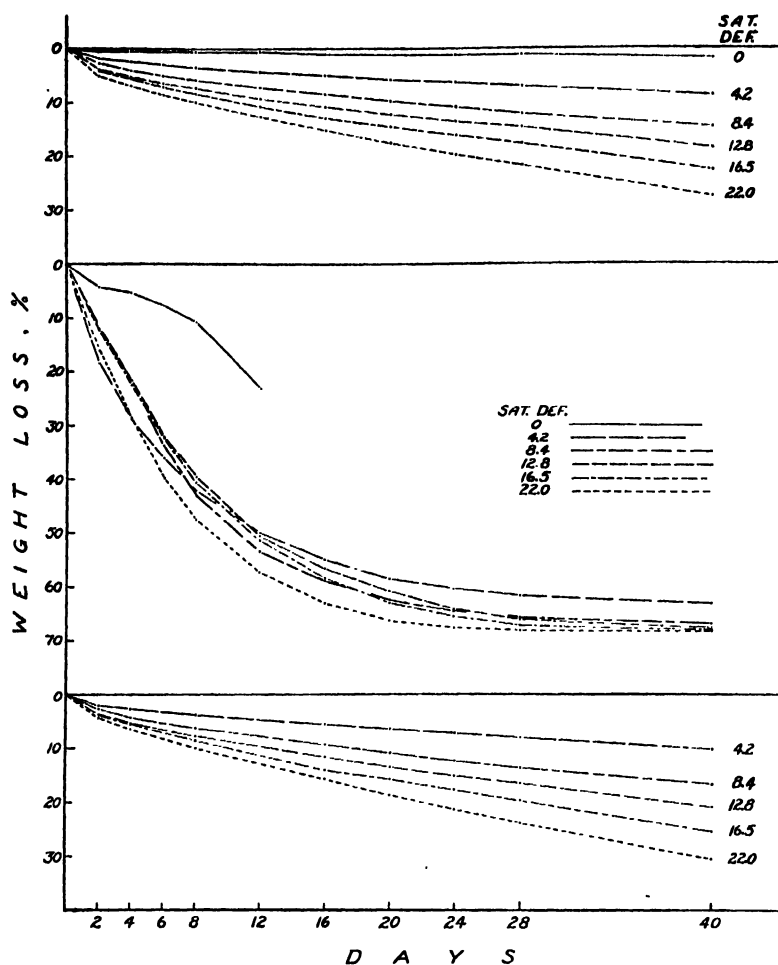


FIG. 1. Percentage loss of weight (water) of cocoons and naked eonymphs in relation to time and saturation deficiency at 75° F. Top panel, sound cocoons with contained eonymphs; middle panel, naked eonymphs; bottom panel, loss from eonymphs within sound cocoons (see text).

Part of the water loss from sound cocoons relates to moisture in the cocoon wall, and the balance to moisture from the contained larva. It is possible to deduce the amount attributable to the larva alone, using the method of Ulllyett. For each determination of gross weight loss, there was a corresponding determination of loss from the empty shells, and since the latter constituted 13.8% of the initial gross weight, the proportion of gross weight loss attributable to shells, and hence also that attributable to larvae, can readily be calculated. The following example, for 12.8 mm. saturation deficiency and a period of 40 days, will make the method clear.

Loss of initial gross weight = 18.64%

Loss of initial cocoon shell weight = 4.92%

$$\begin{aligned}
 \text{Loss from shells} &= 4.92 \times 0.138 \\
 &= 0.68\% \text{ of initial gross weight} \\
 \text{Balance of gross weight loss attributable to contained larvae} \\
 &= 18.64 - 0.68 \\
 &= 17.96\% \text{ of initial gross weight} \\
 \text{This loss, in terms of initial larval weight} &= 17.96 \div 0.862 \\
 &= 20.83\%
 \end{aligned}$$

The estimated water loss from the protected larvae in cocoon samples at saturation deficiencies of 4.2 to 22.0 mm. and for periods of 2 to 40 days, is shown graphically in the bottom panel of Fig. 1. The percentage loss from protected larvae was at first less than the percentage loss of gross weight, but after four to eight days the relation was reversed. This was of course due to the early withdrawal of all moisture from the cocoon wall, all subsequent loss arising solely from the larvae and being naturally a larger proportion of larval weight than of gross weight. The regression of water loss on time for the protected larvae was also slightly curvilinear.

It is also of interest to enquire into the relation between water loss and saturation deficiency for a given time interval. The determined water loss from sound cocoons is plotted against saturation deficiency for four time intervals in the top panel of Fig. 2; the bottom panel contains similar data for the estimated water loss from the contained larvae. Although the plotted linear regression lines (from the formula, $Y = a + bX$) give a fairly good fit for saturation deficiencies between 4.2 and 22.0 mm., the true regression is slightly curvilinear, passing through the origin.

The curvilinearity of the regression, first, of water loss on time at a given saturation deficiency, and second, of water loss on saturation deficiency for a given time, indicating a gradually declining water loss in relation to period and degree of drying, may be due to a decrease in permeability of the cocoon wall consequent upon drying. It can hardly be due to any intrinsic property of the larvae, since when exposed they lose water rapidly in only moderately dry air.

Although the experimental results confirm the opinion reached by Ulyett that the cocoon is very important in the conservation of moisture (being, in *Gilpinia polytoma*, apparently the only mechanism possessed by the larva for this purpose), they show that the cocooned larva is far from being independent of the external environment. The micro-climate appears to be kept near the saturation point by moisture from the larva, as only this condition can account for the water loss at the most humid environments used in the experiments; the maintenance of the humid micro-climate, however, is accomplished at the expense of the insect, since in dry air the cocoon only delays but does not prevent a continuous loss of water from the tissues.

Gain of Water

Experimental results already described have shown that larvae within the cocoons benefit from water gained at contact only gradually, and to a reduced

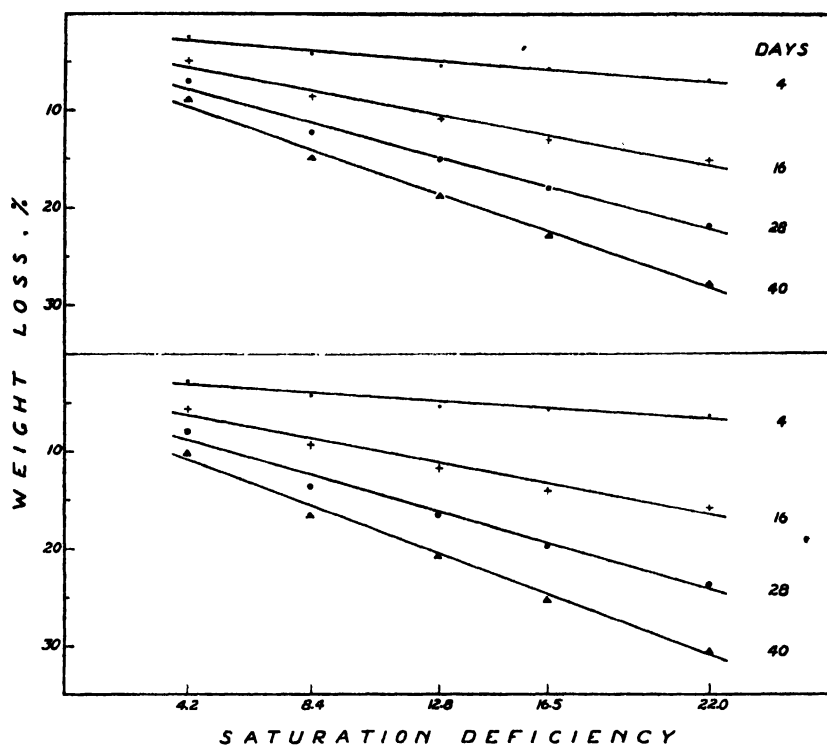


FIG. 2. Regression, weight (water) loss on saturation deficiency, for four time intervals at 75° F. Top panel, sound cocoons with contained onymphs; bottom panel, the contained onymphs alone.

extent or not at all if the water is rapidly lost by evaporation, and that drying reduces either the permeability of the cocoons to water, or the ability of the larvae to benefit from water gained subsequent to drying.

The outer wall of cocoons in contact with water becomes saturated within a short time. In this condition a structure not readily observed in the dry state may be detected, namely, an inner transparent membranous lining (possibly originating as a liquid secretion from the spinning larva, hardening on exposure). The lining is less pervious than the outer wall, and only minute droplets of water penetrate to its inner surface after contact for a day or longer. If wetted cocoons are placed in a saturated environment the minute droplets gradually disappear, and since here the external environment has no evaporative power, one must presume that the droplets are absorbed by the larvae. Two experiments described in the following paragraphs were designed to test this conclusion by means of weight and water content determinations of larvae after immersion of cocoons.

It was impossible of course to obtain consecutive measurements for the same larvae before and after immersion, so that comparisons had to be made between sample lots from a population. The pre-immersion weight of larvae extracted from the cocoons after immersion was deduced by means of deter-

mined relations between gross weight and larval weight. Precautions to reduce errors arising through evaporation from the cocoons and larvae during dissection and weighing, included the use of covered weighing dishes with a very small aperture, and, where only live weights were required, the addition of a non-volatile oil to the weighing dishes, which prevented all moisture loss from the submerged larvae.

The first experiment involved live-weight determinations of larvae extracted immediately after a one- or two-day immersion of the cocoons at 75° F. Some of the cocoons were immersed without previous drying, whereas others were dried (75° F., 22.0 mm. saturation deficiency) for periods of 3 to 56 days before immersion. In all, 28 samples of 100 cocoons each were used; eight samples were opened at the start to determine the initial relationship between gross weight and larval weight, and one sample was opened after each period of drying to determine the then existing relation between gross and larval weight. A summary of the experimental results appears in Table V.

TABLE V

RATIO OF LARVAL WEIGHT TO GROSS WEIGHT, AND LOSS OF WEIGHT (WATER) FROM CONTAINED LARVAE IN RELATION TO PERIOD OF DRYING; COMPARISONS OF THE DETERMINED LARVAL WEIGHTS OF DIFFERENT SAMPLES; AND COMPARISONS OF THE DETERMINED LARVAL WEIGHT AFTER IMMERSION WITH THE DEDUCED LARVAL WEIGHT BEFORE IMMERSION, FOR INDIVIDUAL SAMPLE LOTS

Period of drying in days	0	3	7	14	28	42	56
1. Larval weight as percentage of gross weight	84.92	86.51	86.12	85.21	84.23	83.40	82.19
2. Estimated weight loss from larvae, %	0	4.24	7.67	11.84	20.72	25.64	32.51
3. Determined average larval weight:							
a. Samples not immersed	6.28	5.96	5.70	5.48	4.90	4.70	4.31
b. Samples with 1-day immersion	<u>6.19</u>	5.98	5.91	5.58	5.06	<u>4.64</u>	<u>4.31</u>
c. Samples with 2-day immersion	<u>6.32</u>	6.03	5.92	<u>5.44</u>	5.09	<u>4.67</u>	<u>4.21</u>
4a. Samples with 1-day immersion:							
Estimated weight before immersion	6.19	5.93	5.79	5.52	4.94	4.64	4.30
Determined weight after immersion	<u>6.19</u>	5.98	5.91	5.58	5.06	<u>4.64</u>	4.31
4b. Samples with 2-day immersion:							
Estimated weight before immersion	6.28	5.98	5.81	5.41	4.93	4.67	4.24
Determined weight after immersion	<u>6.32</u>	6.03	5.92	5.44	5.09	<u>4.67</u>	<u>4.21</u>

NOTE: Samples whose average weight is underlined show no evidence of an increase as a consequence of immersion.

A simple method of estimating the effect of immersion is to compare the weights of various sample lots, as in Panel 3 of Table V. There is evidence of gain of water consequent upon immersion in eight of the 10 samples in which weight loss (water) during the earlier drying had not exceeded 21%.

This method is obviously crude since it ignores known differences in original gross weight of the various samples.

A more accurate estimate is obtained by deducing larval weight in a particular sample immediately before immersion. This may be done in two ways, as illustrated for the sample immersed for one day after a 3-day period of drying.

$$(a) \text{ Average initial gross weight} = 7.275 \text{ cg.}$$

$$\begin{aligned} \text{Average initial larval weight} &= 7.275 \times 0.8492 \\ &= 6.18 \text{ cg.} \end{aligned}$$

$$\text{Loss of weight during 3-day drying} = 4.24\%$$

$$\text{Therefore, larval weight prior to immersion} = 5.92 \text{ cg.}$$

$$(b) \text{ Average gross weight after drying} = 6.87 \text{ cg.}$$

$$\begin{aligned} \text{Ratio, larval weight to gross weight after 3-day period of drying} \\ &= 86.51\%. \end{aligned}$$

$$\text{Therefore, larval weight prior to immersion} = 5.94 \text{ cg.} \quad \cdot$$

The average of the two estimates, 5.93 cg., is the best approximation of the true larval weight of this sample prior to immersion. All estimated larval weights in Panels 4a and 4b of Table V have been calculated in this manner. Nine of the 10 samples in which weight loss during earlier drying had not exceeded 21%, showed evidence of a gain during immersion.

It is of interest that immersion for two days had no appreciably greater effect, as measured by these methods, than immersion for a single day. Both failed to compensate at once for a previous loss as low as 4.2% of the initial weight.

The second experiment involved water content determinations of eonymphs in samples analysed immediately before and after, and periodically after immersion for two days at 74° F. Samples analysed at successive intervals after immersion were held meanwhile at 100% relative humidity. Four replications each with over 500 eonymphs, were carried out to determine whether the capacity of the larvae to absorb water was influenced, first, by preliminary drying, and second, by the duration of cold-rest. The data (Table VI) lead to the following conclusions: (1) The water content of eonymphs extracted from cocoons immediately after immersion was not consistently different from that prior to immersion; (2) the water content in samples analysed some days after immersion was in all replications greater than that prior to immersion, and in three of the four replications was greatest in the latest sample after immersion, indicating that absorption of water was gradual; (3) there was no evidence of a relation between duration of cold-rest and ability to absorb water; and (4) eonymphs that had lost about 8 to 9% of their contained water during earlier drying failed to compensate for this loss during two days' immersion and 16 days' storage at 100% relative humidity.

TABLE VI

RATIOS OF WEIGHT OF WATER TO DRY WEIGHT IN SAMPLES OF EONYMPHS EXTRACTED FROM COCOONS BEFORE AND AFTER IMMERSION FOR TWO DAYS AT 74° F.

Replication	1*	2**	3**	4**
Date started	Nov. 21	Dec. 6	Feb. 6	April 7
Initial determination	1.854	1.844	1.868	1.860
Determination after drying	1.703	—	—	—
Determination directly after immersion	1.701	1.863	1.857	1.878
Determination after:				
a. 2 days at 100% R.H.	1.744	1.884	1.939	1.918
b. 4 days at 100% R.H.	1.754	1.862	1.908	1.901
c. 8 days at 100% R.H.	1.751	1.898	1.902	1.898
d. 12 days at 100% R.H.	1.737	1.902	1.900	1.890
e. 16 days at 100% R.H.	1.766	1.948	1.930	1.937

* All samples in the first replication except those analysed in the initial determination, were partially dried for one week at 18% relative humidity, 74° F., before being immersed.

** The other three replications differed from the first only in the absence of a period of drying.

Ecological Significance.

The cocoon, though indispensable for the conservation of the water supply of the contained eonymph, merely delays the outward diffusion of moisture that occurs in unsaturated environments. The final result for the larva is essentially the same as for many entirely unprotected species in which water loss is proportional to saturation deficiency (1). The inner membranous lining of the cocoon is instrumental in preventing flooding of the microclimate. The space within the cocoon does not become flooded even after several weeks' immersion, and though death from suffocation may result in a week or so at 75° F., eonymphs can survive immersion for several weeks in cold water. Moisture penetrating through the cocoon wall is gradually absorbed by the eonymphs, but may also be very quickly lost by evaporation into the surrounding atmosphere. Preliminary drying of the cocoons may cause a greater water loss than can be compensated for by immersion and a subsequent period of two weeks in saturated environment.

The ecological significance of these facts relates to the need for contact water and a fairly extended period during which the natural habitat is at or near the saturation point, following the period of cold-rest, in order that the maximal development in the overwintered cocoons may be realized. The absence of soil moisture, especially if accentuated by unusually warm weather in the spring or early summer, may have consequences on seasonal development extending well beyond the first rainfall.

Field Studies

Temperature

Sample lots of cocoons collected in central Gaspé in the spring, soon after the disappearance of the snow, were placed for the seasonal development in various sites representing the full range of variability in temperature condi-

tions available in the Gaspé forest. Experimental results for three series are shown in Table VII. Although speed of development was clearly influenced by temperature in each location, as evidenced by the mean dates of adult emergence, in no case was there a significant difference in the percentage of development within the samples from a common population.

TABLE VII

PERCENTAGE OF DEVELOPMENT AND MEAN DATE OF EMERGENCE IN SAMPLES OF THREE OVER-WINTERED GASPÉ COCOON POPULATIONS PLACED UNDER DIFFERENT CONDITIONS IN THE FOREST FOR SEASONAL DEVELOPMENT

Year	Forest conditions	Mean Temperature*	Number of cocoons	Development, %	Mean date of emergence
1933	Forest floor, valley bottom	?	288	32.6	Aug. 3
	Forest floor, western slope	48°	289	33.2	July 28
	Seven feet from ground, in shade	55°	296	31.1	July 7
1934	Basement, log building	49°	596	10.2	July 25
	Forest floor, western slope	49 to 50°	580	11.2	July 22
	Seven feet from ground, in shade	53°	583	10.1	July 6
1935	Basement, log building				
	a. Moist sand	52°	402	14.2	July 16
	b. Atmospheric humidity	52°	248	16.5	
	Forest floor, western slope				
	a. Moist sand	53°	387	15.0	July 12
	b. Atmospheric humidity	53°	412	15.5	
	Four feet from ground, in shade				
	a. Moist sand	59°	408	15.7	July 3
	b. Atmospheric humidity	59°	412	16.5	

* Mean temperature in actual location of cocoons during June and July.

An experiment involving a much wider difference in temperature was conducted in 1938. Cocoons collected in two localities in central Gaspé were divided into samples for seasonal development, first, in the natural habitat where mean temperature during June-July was 52° F., and second, in the incubator at 74° F., 100% relative humidity. The results follow:

Locality	Collection date	Location of seasonal development	Number of cocoons	Development, %
Berry Mt. Brook	June 2	Forest floor	770	7.1
	June 13	Forest floor	625	5.3
	June 13	Incubator	434	14.1
Brandy Brook	June 2	Forest floor	917	8.9
	June 13	Forest floor	752	6.5
	June 13	Incubator	380	15.5

Development in the incubator samples was significantly higher than in the field samples, by about 8% on the average, a rather small difference in view of the 22° temperature difference between the two environments.

The results of the field experiments, considered in conjunction with those of the laboratory experiments already described, warrant the conclusion that variations in temperature within the limits normally experienced in the natural habitat of the cocoons have no direct influence upon the degree of development in overwintered cocoon populations.

Moisture

Investigations on the influence of moisture upon development under field conditions fall into three groups, first, moisture conditions controlled; second, effects of rainfall; and third, analysis of weather conditions associated with abnormal development.

Moisture Conditions Controlled

Sample lots of cocoons collected in central Gaspé in September, 1935, were overwintered in wire "flats" under the moss, fully exposed to moisture in the natural habitat; other samples were overwintered in inverted vials so located under the moss that the cocoons were protected from moisture. Early in June half of the samples from each group were placed on moist sand in covered dishes for seasonal development, and the other half were placed in dishes without moisture. All samples were subject to the same temperature fluctuations throughout the year. The remaining cocoons were opened for analysis in the fall of 1936, and the results shown in the accompanying synopsis are based on the number of living larvae at the start of the 1936 season.

Overwintering conditions	No moisture in summer		On moist sand in summer	
	Number of cocoons	Development, %	Number of cocoons	Development, %
Protected from moisture	724	7.5 (4.4-10.5)	793	17.2 (14.9-19.3)
Exposed to moisture	541	9.8 (4.9-13.2)	530	18.9 (16.0-21.3)

(Values in parentheses indicate the range in the four samples in each series.)

Moisture during the overwintering period had no influence on seasonal development, while contact moisture supplied from early June increased development significantly, by about 9% on the average.

The results of an experiment on the influence of moisture on seasonal development of Gaspé cocoons in 1935, are summarized in the lower part of Table VII. It has already been noted that temperature differences between the three sites had no effect upon the degree of development, and it is equally clear that the addition of moisture during the summer was without any influence in either of the three sites.

Samples of Gaspé cocoons collected from water saturated moss, June 8, 1936, were placed under various conditions of moisture ranging from moist sand to dry air. With the exception of the third sample (forest floor), all lots were subject to air temperature in the spruce forest. Development in the various samples was as follows:

Lot	Moisture conditions	Number of cocoons	Development, %
1	Moist sand	151	41.1
2	100% R.H.	198	36.9
3	Near 100% R.H. (forest floor)	228	28.1
4	80% R.H.	189	30.7
5	39% R.H.	169	26.6
6	15% R.H.	197	28.9

A Chi-square test of independence of the entire series shows that equal variations might have occurred due to chance alone in about 14% of trials. When the test is between Lot 1 as a treated sample, and the combined results

TABLE VIII

THE INFLUENCE OF RAINFALL ON DEGREE OF DEVELOPMENT

Locality	Year	Protected from rainfall		Exposed to rainfall	
		Number of cocoons	Development, %	Number of cocoons	Development, %
Brandy Brook, central Gaspé	1939	941	17.2 (16.6)	1012	29.2 (21.8)
Berry Mountain Brook, central Gaspé	1939	1102	19.5 (18.2)	1070	28.8 (22.7)
Cascapedia, southern Gaspé	1939	497	46.4	514	61.2
Matapedia, southern Gaspé	1939	288	61.8 (56.6)	314	69.4 (61.8)
St. Leonard, northern New Brunswick	1939	231	37.3 (36.4)	196	50.0 (46.4)
Acadia Expt. Station, central New Brunswick	1938	548	26.8 (26.2)	148	70.9*
Acadia Expt. Station, central New Brunswick	1939	758	53.6 (52.5)	865	77.0 (73.4)
Millville, central New Brunswick	1938	736	41.3 (31.8)	3483	92.4 (90.3)
English Settlement, Central New Brunswick	1939	2598	32.5 (31.0)	4775	88.9 (86.4)

* Based on emerged adults only, therefore conservative.

of Lots 2 to 6, as an untreated sample, with development values of 41.1 and 30.3, respectively, the probability of a chance variation of equal magnitude is only about 0.04, giving good evidence that water addition caused increased development. When the test is between Lots 2 to 6, the probability of chance variations of equal magnitude is 0.41, precluding an opinion that the degree of atmospheric humidity was significantly related to the degree of development.

Rainfall

The influence of summer rainfall upon percentage of development was checked by exposing spring collected samples of overwintered cocoons to the rainfall that penetrated through the moss into the wire containers, and by protecting other samples by a waterproof cover. Experiments were carried out in eight localities in Gaspé and New Brunswick; the results are shown in Table VIII.

Rainfall data for the localities during the period of development are included in the synopsis.

	May	June	July	Aug.	Sept.
Central Gaspé, 1939	—	3.04	10.25	10.64	4.19
Northern New Brunswick and southern Gaspé, 1939*	—	3.3	6.8	5.6	5.1
Central New Brunswick (Fredericton), 1938	4.42	3.83	5.89	3.32	4.77
1939	2.42	2.00	2.70	1.04	3.89

* *Average of six weather record localities.*

Two values appear under the percentage development in Table VIII. The higher value, including young pronymphs whose reactivation occurred late in the season, though accurate as a measure of the number of individuals overcoming diapause, is somewhat too large as a measure of effective development during the season. A more accurate expression of the latter is given by the value in parenthesis, from which young pronymphs found in the autumn have been excluded. On the basis of the latter values, effective development in central Gaspé in 1939 was increased significantly, by about 4 to 5%, due to the heavy rainfall during the summer. The results for Cascapedia, Matapedia, and St. Leonard were suggestive of the influence of rainfall in increasing development, but the samples were rather small and the differences without statistical significance except in the case of the Cascapedia samples. The four series in central New Brunswick gave large and significant differences in development due to rainfall during the season, even though the rainfall was less abundant than that experienced in the more northerly localities where the effects on development were of a lower order.

Weather Associated with Abnormal Development

Three instances of abnormal development falling within our experience warrant a brief description.

Phenomenally low emergence, of only 1 to 1.6% as determined from records and analysis of over 6300 field collected cocoons, occurred at Parke Reserve, Kamouraska County, Que., in 1934. The emergence occurred between mid-June and late July, and there was no resumption of development among the eonymphs in diapause later in the season. Partial weather records for Parke Reserve, and complete data for the nearest permanent station of the meteorological service (Ste. Anne de la Pocatière) appear in the synopsis.

	May	June	July	Aug.
Parke Reserve				
Mean temperature	—	56.3	61.2	57.5
Rainfall	—	4.67	3.29	2.92
Ste. Anne de la Pocatière				
Mean temperature	52.2	59.0	65.6	61.6
Departure from normal	+2.7	+1.1	+1.0	+0.7
Rainfall	1.85	4.87	2.94	3.77
Departure from normal	-1.38	+1.86	-0.52	+0.45

There was a marked deficiency of rainfall in May, while later in the season rainfall was close to, or above, normal. The deficiency in May, which was associated with high temperature, was accentuated by the open growth and shallow moss layer in the forest, encouraging the rapid drying out of the debris sheltering the cocoons. The failure of the population to respond to liberal moisture in June and later was possibly due in part to consequences of the earlier drying, though it was typical of the normal behaviour in a one-generation area.

The effect of dry weather in 1938 and 1939 on the overwintered population in the two-generation area in south central New Brunswick was of an entirely different character from that noted above. Normally, emergence from the overwintered cocoons in this region is completed in early July, but in 1938 and 1939 there was a period of heavy emergence in late May through June, and a straggling emergence lasting until October. Many partially developed insects were still inside the cocoons in the autumn. The total seasonal development, however, approximated that which more typically occurs from May to early July.

Climatic data for the two seasons follow (Fredericton records).

	May	June	July	Aug.	Sept.
1938—					
Mean temperature	49.3	64.3	67.1	67.2	55.9
Departure	-1.6	+4.1	+1.1	+3.1	-0.1
Rainfall	4.42	3.83	5.89	3.32	4.77
Departure	+1.29	+0.13	+2.33	-0.66	+1.27
1939—					
Mean temperature	50.0	59.5	67.6	68.6	56.2
Departure	-0.9	-0.7	+1.6	+4.5	+0.2
Rainfall	2.42	2.00	2.70	1.04	3.89
Departure	-0.71	-1.70	-0.86	-2.94	+0.39

The monthly summaries for 1938 show no apparent cause of protracted development, since rainfall was abundant during the early summer months. Actually, over 95% of the May precipitation occurred during the first three weeks when air temperature was low and when soil temperature in the spruce woodlands was almost continuously below the threshold of development. Following this there was a period of unseasonably warm weather with only 1.78 in. of rainfall from May 22 to June 23, the period at which development within the overwintered cocoons generally is most active.

In 1939 there was a scarcity of rainfall in south central New Brunswick from May until September. Moreover, the May rainfall was of little benefit to the sawfly since it occurred when the soil temperature was below the threshold of development practically every night, and above it to the extent of only a few degrees for a limited period during the warmest part of the day. The forest floor was very dry during the gradual rise of soil temperature in June.

Experiments already described leave no doubt that the gradual response of eonymphs during the summer and early fall was due to the effect of delayed rainfall. It is of interest to note that while development in the absence of contact water in the natural habitat remained low, high development resulted in samples incubated without contact water at 74°, 100% relative humidity (e.g., 32.5 and 98.5% development, based on 2598 and 623 cocoons, respectively, in English Settlement samples). The constant high temperature of the incubator therefore provided a stimulus to development in this two-generation material equivalent to that provided in nature by precipitation during the summer.

Reviewing the evidence regarding the influence of moisture, it is fairly clear that moisture conditions during the winter months, or prior to the spring rise in temperature above the threshold, have little effect upon seasonal development in the natural populations. Moisture deficiency at the time of normal spring development is likely to have adverse effects, which, however, are different in one-generation and in two-generation areas. In a two-generation area many of the overwintered eonymphs that fail to develop at the normal time respond to moderate rainfall later in the season, so that the chief result of dry spring weather is a protraction of the emergence period. As for one-generation areas, although continual contact with moisture from early June increased development by about 9 to 10% in two experiments, and although excessive rainfall in July and August of 1939 increased development in central Gaspé populations by 4 to 5%, the bulk of the evidence from studies in central Gaspé and at Parke Reserve shows that the overwintered populations typically fail to respond in any perceptible degree to moisture addition after mid-June. The effect of unseasonably dry conditions in the spring is therefore to reduce still further the characteristically low development in the overwintered populations in a one-generation area.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

NOVEMBER, 1941

NUMBER 11

THE DETECTION OF CORAMINE¹

BY G. H. W. LUCAS²

Abstract

Methods are described for the biological detection of coramine and for the production of coramine crystals with mercuric chloride. By the use of the crystal method as small an amount as 0.004 mg. of coramine has been detected.

This Laboratory has had to undertake the examination of horse saliva for the detection of various drugs used as stimulants and from the first has found pharmacological tests of great value. In the routine testing for strychnine, small frogs were injected with a purified extract of horse saliva. In some frogs it was noted that such an extract, which subsequently was proved not to contain strychnine, produced a peculiar type of convulsion and at times death. Suspicion was directed to coramine. Small young frogs were tested with small amounts of this drug and convulsions identical with those produced by the saliva from horses were observed.

Consequently, in the racing season of 1936 several experimental horses were given stimulant doses of this drug by mouth and a saliva sample was obtained. Later, when it was suspected that the drug was being given hypodermically to horses, the drug was administered in this way and again the saliva was examined after purification in the following routine manner.

Extraction of Coramine from Saliva

To the saliva and the washings of the swabs used in the collection of the sample, glacial acetic acid was added in an amount necessary to produce a concentration of 5% acetic acid. The mixture was then heated to 65° C. to precipitate some of the protein, and this was filtered out. The acid and the water were removed from the filtrate by heating *in vacuo* at 65° to 70° C. The dry residue was extracted five or six times with 10 to 12 cc. of boiling absolute alcohol. The combined extracts, centrifuged clear of protein, were warmed with a drop of 70% sulphuric acid, causing more protein to precipitate; this was removed by centrifuging. The supernatant liquid was evaporated to dryness on a steam bath under a fan. From the residue, taken up in about 1 cc. of water, the fatty material was removed by filtration through a micro filter made of asbestos. The filtrate, made ammoniacal,

¹ Manuscript received June 2, 1941.

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was extracted at once with 3 to 4 cc. of a mixture of 8% chloroform in ethyl acetate. Two or three extractions were made, and the combined extracts were evaporated to dryness on a steam bath. The residue was dissolved in $\frac{1}{2}$ to 1 cc. of *N*/20 hydrochloric acid, the solution was made ammoniacal and re-extracted with pure chloroform. The chloroform extracts were evaporated on a steam bath and the residue was again taken up with a few drops of *N*/20 hydrochloric acid.

One portion of the final extract was examined by injecting it into a small frog after the hydrochloric acid was neutralized with sodium bicarbonate, while another was mixed with bichloride of mercury and set aside for crystalline tests. It was found that doses of coramine (given by mouth or subcutaneously), which produced stimulation in the horses, appeared in the saliva in amounts sufficient to cause convulsions in a small frog and to give crystals with bichloride of mercury. Hence the following experimental work was undertaken.

Pharmacological Test for Coramine

Using a fine hypodermic needle, varying amounts of coramine were injected through the floor of the mouth, into the abdominal lymph sac of frogs about 1 gm. in weight. About 1 mg. of coramine per gm. of frog produced the following marked reaction. A few minutes after injection the fore limbs began to stiffen. They were gradually extended to a right angle with the body, and the point of the chin fell to the supporting surface, a wetted porcelain plate. The pupils contracted and the eyeballs were withdrawn. The frog either died in about half an hour or eventually recovered. If a smaller dose (0.2 to 0.3 mg. per gm. of frog) was given, the frog after several minutes to a quarter of an hour appeared normal but, if stimulated by touching the point of its nose with a sharp point (needle) or by drawing such an instrument down its vertebral column, the front limbs were gradually extended stiffly and the above sequence of events occurred. Figs. 1, 2, and 3 show the reaction.

It was found that certain small frogs of about 1 gm. weight, when stimulated, reacted in the typical manner to 0.2 mg. of the drug. In most frogs weighing up to 30 gm., the response occurred with 0.4 to 0.5 mg. per gm. Without stimulation, all such frogs gave a typical marked response with 0.6 mg. per gm. or more. The reaction was produced in frogs 1 or 2 gm. in weight by a smaller amount of coramine per gm. than was required for large frogs.

Crystalline Test for Coramine

About the time that the pharmacological test for coramine was being developed, Mr. Charles Morgan, Chemist of the New York State Racing Commission, had discovered that coramine gave typical crystal forms with bichloride of mercury and he was good enough to write in regard to this. In this laboratory Mr. Morgan's observations have been confirmed.

Either with the extract from saliva prepared as indicated above, or coramine itself, typical crystals whose forms are shown in Figs. 4, 5, and 6 can be



EXPLANATION OF FIGURES

FIG. 1. A frog in the early stages of coramine poisoning; the pen point touching the nose caused the convulsion to appear.

FIG. 2. The frog after convulsions are produced. This position is maintained for 10 min. or more, unless the frog is disturbed.

FIG. 3. The frog in the final stages of poisoning.

FIGS. 4 AND 5. Coramine crystals (sheaf-like) near bichloride of mercury crystals ($\times 30$).

FIG. 6. A sheaf of coramine crystals ($\times 80$).

produced. If a drop of fluid containing 0.4 mg. of coramine or less is allowed to mix on a slide with a drop of a 5% solution of mercuric bichloride and evaporation is allowed to occur, the development of many typical crystals occurs. This first occurs towards the margin of the drop. Crystals of mercuric bichloride form and a little later, usually inside the ring of bichloride, the feathery crystals of the coramine mercuric compound develop. Their characteristic form is shown in the figures.

Acknowledgment

The author wishes to express his thanks to Prof. V. E. Henderson for his suggestions, advice, and criticism while this work was in progress.

THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

III. BIOCLIMATIC RELATIONS^{1,2}

BY M. L. PREBBLE³

Abstract

The progress of intracocoon development in relation to temperature and moisture is described for a one-generation and a two-generation area in eastern Canada. In the one-generation area (central Gaspé) the degree of seasonal emergence from the overwintered cocoons can be forecast with considerable accuracy by means of sample analyses during the period of pronymphal and early pupal development. This is possible since few individuals that have not initiated development by late June do so later in the summer, even though environmental conditions are quite favourable. The technique fails in a two-generation area (south central New Brunswick) because members of the overwintered population may continue to respond to favourable temperature and moisture conditions throughout the entire season.

On the basis of biologic and climatic data the area occupied by the European spruce sawfly in eastern North America is divided into zones representing the probable distribution of one-, two-, and three-generation areas; intermediate transitional zones are also indicated.

The spruce sawfly encounters and has shown its adaptability to a considerable variety of climatic conditions throughout its distribution range in North America, producing a variable number of annual generations through variations in the diapause behaviour. The principal facts relating to seasonal development and climate, and a tentative division of the area occupied, or likely to be occupied in the future, into zones according to the number of seasonal generations, are outlined in this paper.

Climate and Development in a One-Generation Area

Climate

Most of the studies in a typical one-generation area were carried out in the valley of Berry Mountain Brook, one of the headwaters of the Cascapedia River in the Gaspé Peninsula, Que., at an altitude of 1500 feet above sea level. The region is characterized by high ridges, plateaus, and mountains to a height of 3000 feet or more and rather narrow valleys. Owing to the high altitudes and proximity to the sea, the climate is cool and the growing season short. Snow persists in the heavily wooded valley bottoms until late May or early June. Maximal daily temperature occasionally exceeds 75° F. in midsummer but the nights are cool. Freezing temperatures occur commonly up to mid-June and from early September onwards. Rainfall is

¹ Manuscript received in original form March 29, and as revised, July 25, 1941.

This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April, 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Parts I and II of this series appeared in the October issue.

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frequent and abundant, and light snowfalls occur sometimes in September and commonly in October. Average temperature and precipitation data for June to October, based on records in the wooded valley bottom from 1932 to 1939, are summarized in the following synopsis.

	Mean air temperature	Mean soil temperature*	Precipitation, in.**
June	52.9	46.5	3.88
July	57.2	52.8	5.78
August	55.1	53.4	5.42
September	47.0	47.0	4.43
October (first half)	37.7	39.4	1.63

* At depth of about 2 in. in moss.

** Includes rain and snow, the latter as 10 : 1.

Development within the Cocoon

Development in the overwintered cocoons in central Gaspé begins soon after the snow disappears. Pronymphs are most numerous about mid-June, at approximately the same time that buds of white spruce and balsam fir burst. The first pupae appear about mid-June and the first adults late in the month. Emergence is usually heaviest in July though stragglers continue to appear until mid-August or later.

Two methods have been used in following the progress of development in overwintered cocoons. In the first, sample lots of cocoons are collected from the undisturbed moss at successive intervals, for immediate analysis. The method has the disadvantage that emerged sawflies cannot be included in the analysis, owing to the impossibility of distinguishing between empty cocoons of the current season and accumulated ones of former years. Consequently the highest percentage values of development (all stages beyond the eonymph) are obtained before adult emergence commences; the values drop as emergence progresses and approach zero when emergence is over for the season. Results of analyses in 1932 and 1935 appear in Table I.

The 1932 series indicated about 13% development in the stand under observation, an estimate corroborated by emergence of 220 adults (12.2%) from 1806 cocoons from the same stand kept for seasonal emergence. The estimated development from the 1935 series was about 26%, which was checked by 22.4% emergence from 2259 cocoons kept for seasonal emergence.

The second method consists of dividing a large collection of cocoons made late in the spring into sample lots of about 200, each of which is placed in a shallow wood and wire container under the moss. Successive lots are removed for analysis at intervals of two or three days, and, since adults and emerged cocoons are retained, the progress and percentage of development can be compared for all lots in the series. Data from extensive series studied in

TABLE I
ANALYSES OF PERIODIC COCOON COLLECTIONS, BLACK SPRUCE SLOPE,
BERRY MOUNTAIN BROOK, GASPÉ

Time of collection and analysis	Number in sample	Pronymphs, %	Pupae, %	Unemerged adults, %	Total development*, %
1932					
June 21 - 27	376	6.7	6.9	0	13.6
July 2 - 5	444	1.8	5.6	1.4	8.8
July 7 - 13	306	2.0	3.6	1.6	7.2
July 18 - 23	356	1.4	2.8	3.1	7.3
July 26 - 29	395	0.8	0.5	0.2	1.5
Aug. 2 - 6	336	0.6	1.5	1.5	3.6
Aug. 8 - 12	251	1.6	0	0	1.6
Aug. 19 - 25	253	0	0.4	0	0.4
Sept. 1	151	0	0	0	0
1935					
June 21	139	7.2	15.8	3.6	26.6
June 28	198	0.5	16.7	5.6	22.8
July 4	206	2.4	5.3	7.3	15.0
July 10	156	1.9	2.6	9.0	13.5
July 19	153	1.3	0.6	0	1.9
July 26	122	0	0	0	0

* *Eonymphs, not shown in the tabulation, are complementary to the percentage of total development.*

1933, 1934, and 1939 are represented graphically, along with temperature and precipitation, in Figs. 1 to 4. In order to simplify the charting, no distinction has been made between living and dead, or emerged and unemerged adults; the final moult being considered the ultimate attainment in the course of development. Eonymphs, being complementary to the percentage of total development, have not been included in the graphs. As the figures are largely self-explanatory, little descriptive comment is necessary. In all but one of the series only eonymphs and pronymphs were present in the first samples analysed; pronymphs increased to maximum about June 15-20, then gave way gradually to pupae and ultimately to adults. Variations in the proportions of adults in the later samples and in the percentage of total development in all but the earliest samples, were entirely of a random nature. Hence the respective curves are indicated as straight lines of no slope at the mean for the series, starting when the characteristic mean value was first attained in individual samples. The curves for pronymphs and pupae decline gradually to the horizontal axis, disregarding the few individuals, most of which were dead, contained in late sample lots. The pertinent statistics for the four series are included in Table II. The slight discrepancy between the curves for adults and total development in each series was due in part to sampling errors (different numbers of sample lots figuring in the calculation

TABLE II

COMPARISON OF STATISTICS BASED ON SAMPLES OF COCOONS ANALYSED PERIODICALLY DURING THE SEASON, AND ON OTHERS HELD FOR SEASONAL EMERGENCE

	1933	1934	1934	1939
Forest type	Black spruce slope	Black spruce slope	White spruce flat	Black spruce flat
Figure showing analyses	1	2	3	4
Total number insects in series	7145	10,864	9913	9735
Number of samples	40	41	38	39
Total development, %	41.0	13.4	25.6	17.8
Basis, samples during period:	June 16 - Aug. 31	June 12 - Aug. 19	June 11 - Aug. 18	June 6 - Sept. 14
Number insects attaining adult stage, %	39.4	12.9	21.4	15.2
Basis, samples during period:	July 21 - Aug. 31	July 21 - Aug. 19	July 28 - Aug. 18	July 21 - Sept. 14
Check from lots held for seasonal emergence:				
Number of cocoons	1454	1759	2498	941
Emergence, %	35.0	11.0	22.8	16.3

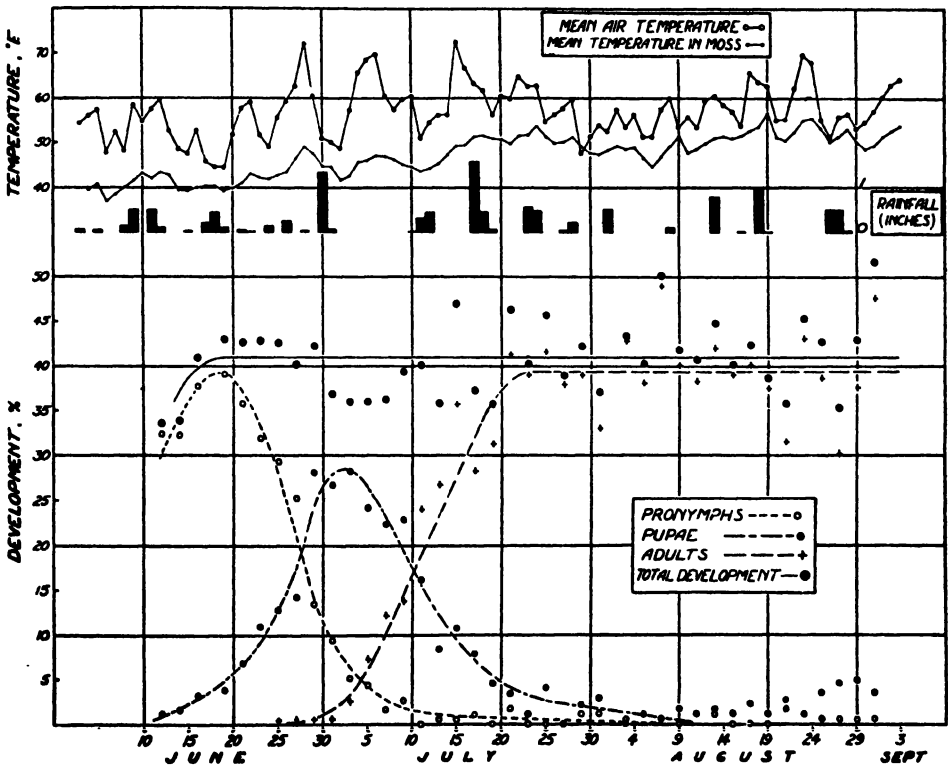


FIG. 1. Progress of development within overwintered cocoons; black spruce slope, Berry Mountain Brook, central Gaspé, 1933.

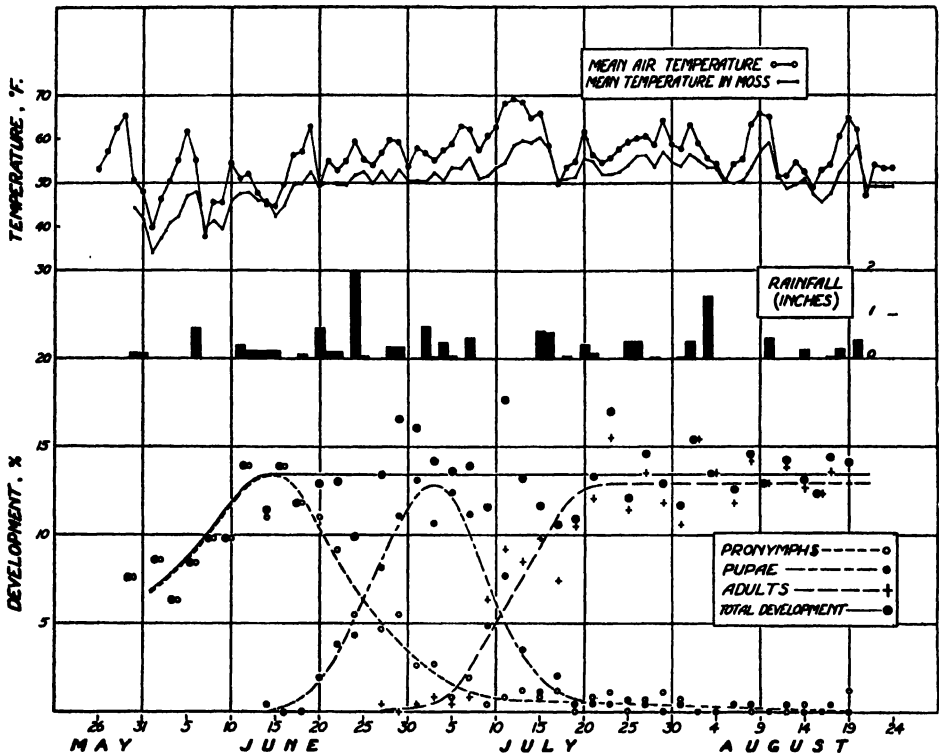


FIG. 2. Progress of development within overwintered cocoons; black spruce slope, Berry Mountain Brook, central Gaspé, 1934.

of the two curves), and in part to the death of a few pronymphs and pupae. The check values obtained from lots held for seasonal emergence (Table II) were very close to those resulting from the periodic analyses.

It is clear from the results of both methods of analysing development in overwintered cocoons, that a reliable estimate of the percentage of seasonal development in Gaspé populations can be obtained two to three weeks before emergence begins, and about five weeks before emergence is near completion for the season. This is so because very few individuals that have not resumed development by the latter half of June respond later in the season even though soil temperature is higher and precipitation abundant during July and August. One must conclude that the resumption of development in the Gaspé populations is controlled by factors operative before mid-June.

There is virtually no tendency for a second generation in central Gaspé even in the warmest seasons. Only two definite records of summer emergence from newly spun cocoons were obtained in eight years' studies, though over 18,000 reared cocoons and many times that number of collected newly spun cocoons were under observation. However, there is occasionally some development of newly spun eonymphs into pronymphs in the late summer, but

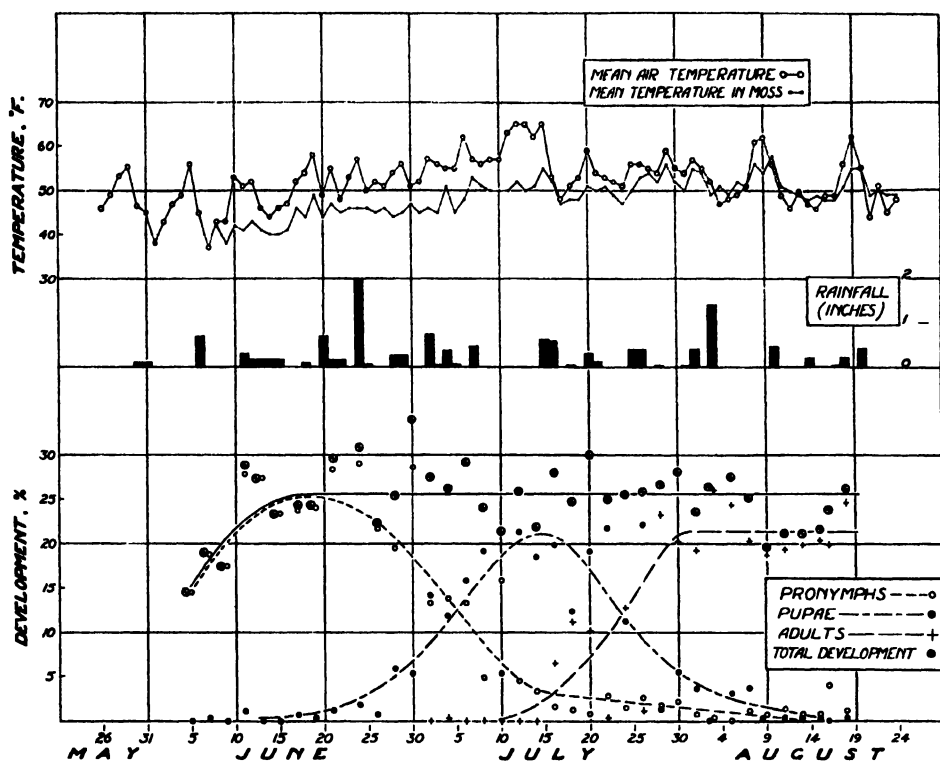


FIG. 3. Progress of development within overwintered cocoons; while spruce flat, Berry Mountain Brook, central Gaspé, 1934.

the pronymphs* either enter into diapause that persists for several months at optimal conditions, or are inactivated by cold, and in either case their further development is impossible until the following spring. In this respect the spruce sawfly differs from *Lyda hypotrophica* Hartig (9) and *Lygaeonematus pini* Retz. (7), which undergo considerable development within the cocoon during the winter.

Climate and Development in a Two-Generation Area

Climate

Studies in a typical two-generation area have been concentrated in the Fredericton district of south central New Brunswick. The region is moderately rolling with broad valleys and low hills, few of which exceed 500 feet above sea level. Snow disappears from the spruce woodlands in late March or April, though occasionally it lasts until early May. Freezing temperatures are infrequent after the last of April, or before early October, and maximal temperatures of 80° F. or higher are common between late May and early September. Normal climatic data for Fredericton are included in the following synopsis.

* These possibly represent emergent strains which, as noted elsewhere, cannot be wholly eliminated in a strictly one-generation area.

	Mean air temperature	Mean soil temperature*	Rain	Snow	Total precipitation
January	13.1	—	1.51	23.6	3.87
February	14.6	—	0.91	23.0	3.21
March	26.0	—	2.01	16.3	3.64
April	38.7	36.2**	2.19	7.0	2.89
May	50.9	42.9	3.11	0.2	3.13
June	60.2	53.3	3.70	0	3.70
July	66.0	58.7	3.56	0	3.56
August	64.1	59.4	3.98	0	3.98
September	56.0	52.7	3.50	0	3.50
October	45.5	43.0	3.98	0.4	4.02
November	32.5	—	3.04	8.0	3.84
December	18.9	—	1.53	19.0	3.43

* Based on five seasons' records taken under the moss in spruce-balsam woodlands.

** Record for April of one year only.

The growing season is close to two months longer, and the mid-seasonal mean air temperature about nine degrees higher, than in central Gaspé.

Development within the Cocoon

Development within the overwintered cocoons in the Fredericton district starts in late April or early May. The first pupae appear about mid-May and the first adults late in the month. Emergence is normally ended by July,

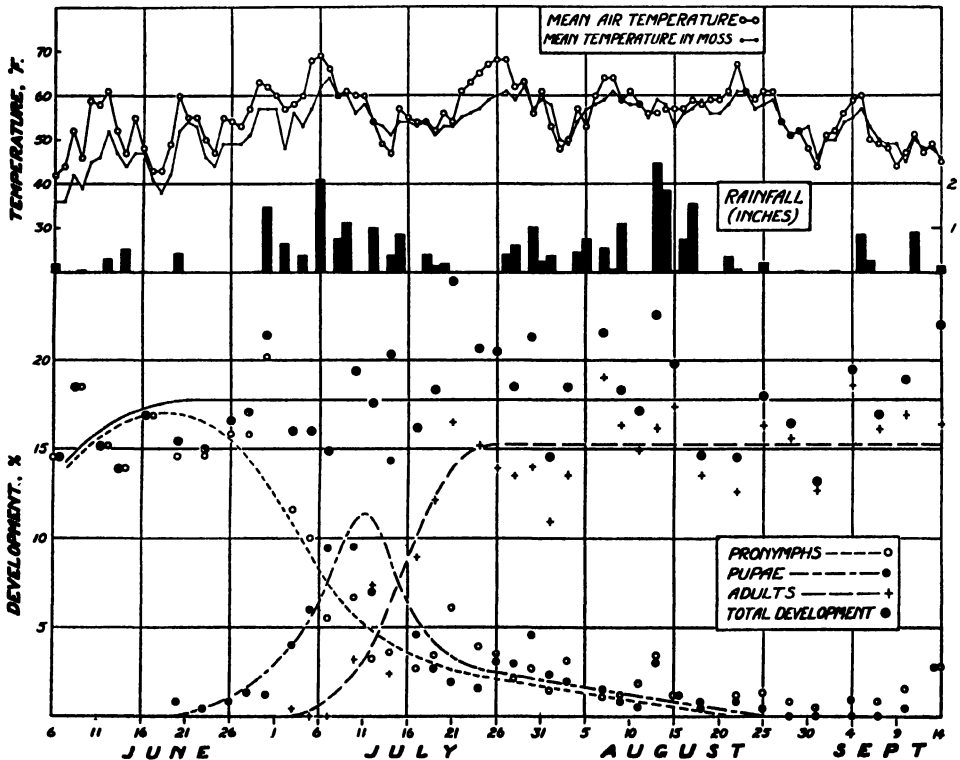


FIG. 4. Progress of development within overwintered cocoons; black spruce flat, Berry Mountain Brook, central Gaspé, 1939.

but in the event of unusually dry spring weather the resumption of development in many of the overwintered cocoons is retarded. Due to the capacity of the overwintered eonymphs to respond to moisture later in the season it is not possible to forecast the percentage of development by the methods so satisfactory for a one-generation area. This is clearly shown by the analyses (Table III) of samples of overwintered cocoons in 1939, a season with dry weather in late May-June, and in late July-August, and with abundant rainfall in September and October. It is significant that many eonymphs resumed development in the latter two months.

TABLE III

PERIODIC ANALYSES OF SAMPLE LOTS OF OVERWINTERED COCOONS, ENGLISH SETTLEMENT, YORK CO., N.B., 1939

Date of analysis	Number in sample	Pronymphs, %	Pupae, %	Unemerged adults, %	Emerged adults, %	Total development*, %
May 14	150	18.7	0	0	0	18.7
May 27	218	19.7	2.8	0	0	22.5
June 4	299	12.1	6.0	0	0	18.1
June 10	285	11.9	12.3	0.7	0	24.9
June 17	295	12.2	10.5	2.4	0	25.1
June 24	304	8.9	11.8	8.6	1.0	30.3
July 1	278	4.7	7.9	1.4	6.5	20.5
July 8	247	6.5	6.1	3.6	7.7	23.9
July 21	312	7.0	7.0	2.6	19.9	36.5
Aug. 6	278	14.4	14.0	8.6	24.5	61.5
Aug. 12	265	9.1	11.3	6.0	26.0	52.4
Aug. 19	307	8.5	3.6	1.6	29.0	42.7
Sept. 1	298	10.4	10.7	4.4	39.3	64.8
Oct. 10	314	30.9	7.3	3.5	35.0	76.7

* Eonymphs, not shown in the tabulation, are complementary to the percentage of total development.

Although a considerable loss in productivity results from dry spring weather, the latter does not prevent the occurrence of a fairly strong second generation, other conditions being favourable.

Zones with Different Numbers of Generations*

In addition to central Gaspé and south central New Brunswick, knowledge of seasonal development is available also for the Parke Forest Reserve, Kamouraska Co., Que.; for the Timiskaming district of Quebec and Ontario, where a partial second generation is produced (1); for southwestern Maine, where a partial third generation may occur (8); and for Connecticut where three generations are produced (4, 5). Information from points distributed throughout the range of the insect in eastern Canada has been obtained from the Forest Insect Survey. These combined records in conjunction

* Dr. A. W. A. Brown, Entomologist-in-Charge, Forest Insect Survey, Division of Entomology, Ottawa, is joint author of this section.

with meteorological data for many stations, permit the division of the occupied and adjacent areas in eastern North America into zones in which a different number of seasonal generations may be expected.

Meteorological data were accumulated for 137 stations in Canada and for 104 stations in the United States, distributed in the area bounded by the 51st parallel of latitude, the 82nd meridian of longitude and the Atlantic Ocean, including the whole of the present known range of distribution of the insect in North America. In view of the relative unimportance of rainfall as a factor influencing the number of generations in areas where most intensive studies have been conducted, the climatic analysis has been restricted to temperature characteristics alone.

The first question to be settled is that of a temperature index satisfactory for the description of each locality. With so many localities necessary for adequate representation of the large areas involved, it has been impossible to deal with daily temperature data; consequently monthly summaries have had to be used although the writers are well aware of their limitations. The temperature index must also be restricted to the developmental season, since winter conditions have no relation to sawfly development. Seasonal mean temperature is unsuitable as an index since it fails to take account of the length of the season. The only reasonable approach is to work out an index including both temperature and time, in the manner so long ago used by Merriam (6) in the enunciation of the laws governing northward distribution of plants and animals. Shelford (10) attacks Merriam's method, which was the accumulation of daily mean temperature above a theoretical threshold, on several grounds: (1), the erroneous summing above 32° F., instead of 43°, which was the theoretical threshold, thus invalidating the conclusions but not necessarily the method; (2), the failure of day-degrees calculated from a daily mean to coincide with or in many cases even to approach values based on hourly or two-hourly means; and (3), the failure to recognize that the effect of one degree of temperature is not necessarily the same at all points of the temperature scale. Moreover, Kendeigh (3) has shown the method to be basically erroneous in relation to birds and mammals, which through regulation of body temperature and modification of the micro-climate (nest temperature in the case of birds) are to a large extent independent of environmental temperature above a threshold or toleration point.

With these considerations in mind, proposals involving the use of accumulations of monthly means above a given base are made with full realization of the lack of scientific precision, though the procedure is not without precedent. (Hopkins (2) uses the method for arriving at the sum of effective temperature for record localities in bioclimatic studies.) The justification for the use of the method in the present instance is that deductions based on analysis of climatic data are in accord with the facts in a number of localities for which biological data are available.

The same threshold, viz., 43° F., as that employed by Hopkins has been used here. This is very close to the threshold value for development within the

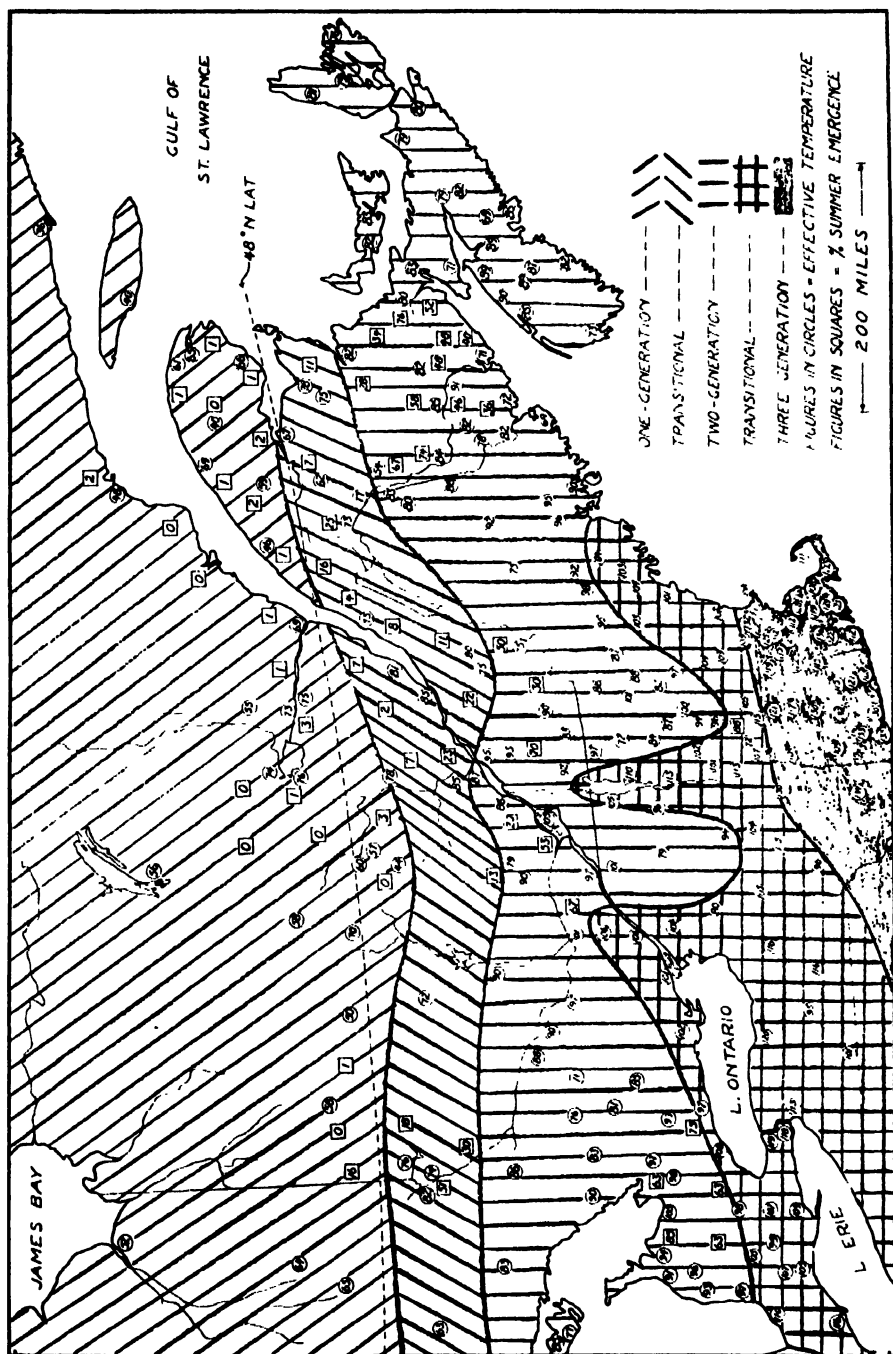


FIG. 5. Map of northeastern North America showing division into zones according to number of seasonal generations. (Method of zonation described in text.)

cocoon, for incubation, and for larval feeding though admittedly a monthly mean of 43° is an abstract value. Monthly mean temperature above this base was accumulated for each locality for which climatic data were obtained, and the sums are shown on the accompanying map (Fig. 5).

The use of the sums of "effective monthly temperature" in zoning areas characterized by variable numbers of seasonal generations of the spruce sawfly rests on the following argument.

1. The sum for central Gaspé based on monthly normals is 40; based on a very favourable year (1937) it is 49; and based on a very unfavourable year (1936), but one with survival of a small proportion of the larval population, the value is 33.

Other areas known to have but a single generation, or at most an entirely insignificant emergence for a second generation not normally surviving, have somewhat higher sums: Parke Reserve, 60; Causapscal, 59; Gaspé Harbour, 65.

2. The sum for the Fredericton district based on monthly normals is 85; based on a very favourable year (1937), 101; and based on a very unfavourable year (1936, small second generation), 79.

The sum for various localities in the Timiskaming district and in northern New Brunswick, known to have a small second generation, averages about 72 to 73.

3. The sum for the three-generation area in Connecticut, based on nine localities in the State, averages about 124.

Extensive biological data are also available to aid in the zoning of areas characterized by variable numbers of seasonal generations. Although intensive field studies have been carried out in only a few localities, records on developmental behaviour have been obtained from survey samples collected throughout the entire range of distribution in Canada. The studies in south central New Brunswick have shown that the earlier cocoons of the first generation yield a high summer emergence, later ones practically none, even though temperature conditions are not sensibly different at the time of spinning. This suggested the possibility of characterizing Canadian localities from which sample lots originated, as one-generation, two-generation, or transitional areas (the latter with a small second generation, relatively unimportant), by analysis of emergence in survey samples received by the Forest Insect Survey. The analysis has been restricted to those samples containing cocoons spun by early August, except in the case of samples originating in northern areas (the 48th parallel of latitude has been taken as an appropriate demarcation) where development is so much later that very few or no cocoons are spun by that time. Consequently the analysis of samples from northern areas has been extended to all samples of the season. The percentage values of summer emergence shown on the map (Fig. 5) are based on the pooled results of four years' survey records at Ottawa, one year's records at Fredericton, and one year's records at Duchesnay, Que. Owing to the light infestations throughout

much of the distribution range, and the consequent small samples, results for contiguous localities (within a radius of about 35 to 40 miles) have been combined. The dependability of the calculated values, as a measure of the developmental behaviour of the populations from which the samples arose, can be evaluated in a number of instances.

1. Emergence values of 1 to 2% were obtained for samples from the coastal areas of the Gaspé Peninsula. Although a second generation does not occur naturally, it is not unexpected that a small emergence would result in material reared during the latter part of the larval development at the more favourable climatic conditions at the Forest Insect Survey headquarters. In fact, the results almost exactly duplicate those reported in a previous section, where continuous rearing of central Gaspé stock, from the egg stage onwards, at Fredericton gave a 1% emergence value.

An emergence value of 4% was obtained from samples originating in Kamouraska County, Que., known to be an area in which one generation is the rule, with occasionally a very small partial second generation.

2. Although results for individual localities in the Timiskaming district were quite variable, due partly to small samples, the combined data for all localities and years indicate an emergence value of about 33%, corresponding closely to Dr. Atwood's field determination of 30%.

3. Emergence values of 7 to 25% were obtained for samples from northern New Brunswick, known to have only a partial second generation; and of 28 to 76% for samples from central and southern New Brunswick, where a strong second generation is the rule.

The various zones were defined as follows:

One-generation zone: sum of effective temperature not exceeding 65; emergence value not exceeding 3%.

Transitional zone: partial second generation normal or occurring in exceptionally favourable seasons: sum of effective temperature, 66 to 80; emergence value usually under 25%.

Two-generation zone: sum of effective temperature, 81 to 100; emergence value usually over 40%.

Transitional zone: partial third generation: this zone is established only tentatively, based on the probability that a partial third generation occurs in areas climatically similar to southwestern Maine; sum of effective temperature, 101 to 115.

Three-generation zone: sum of effective temperature, in excess of 115.

The zone boundaries shown on the map (Fig. 5) were established by interpolation, an attempt being made to strike a balance between climatic and biologic data where these were not in accord. A few examples will suffice to explain how this was done. A fairly high sum of effective temperature, i.e., 88, indicative of a two-generation area, is characteristic of the Berthier district of Quebec, while the emergence value based on survey samples (20%)

is not sufficiently high to warrant the classification of the district as a two-generation area. But since the samples were not extensive, greater weight was given to the climatic data. A few examples where the climatic data were not used in the final zonation include, (*a*), Sidney, Saint John, and Eastport, coastal towns with effective temperature sums of 76, 71, and 69, respectively, which are dissimilar to localities only a few miles inland; (*b*), large cities such as Quebec and Montreal, with effective temperature sums of 85 and 105, because summer conditions in large cities are known to be non-representative of the surrounding region; and (*c*), localities of exceptional temperature sums compared with neighbouring points, due to altitude or other local features, e.g., Plaster Rock, Victoria County, N.B., with an unusually low sum of effective temperature, i.e., 54, for an area known to be a two-generation area. Other discrepancies may also be noted, since it is obviously impossible to attempt a classification of the large area except on the basis of regionally characteristic indices.

It is of especial interest to point out that the Saguenay valley of Quebec was originally classified as a transitional zone in which a partial second generation may be expected. This zonation was supported by the (normal) climatic indices, and by the biologic data prior to 1940. The biologic data based on more extensive samples in the unfavourable season of 1940 were not indicative of a partial second generation, and were of sufficient weight to depress the average emergence value to 3% or less. Therefore this area is not indicated as a transitional zone in the present classification, though it is possible that in reality the area is transitional in the strict sense, having a partial second generation only in the most favourable seasons.

The writers anticipate that corrections in the zonation will probably be necessary as knowledge of the regional behaviour of the spruce sawfly increases. In the meantime the proposed zonation will serve to focus attention upon areas doubtfully classified at present, and upon various aspects of the bioclimatology requiring further study.

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LA DIAPAUSE CHEZ LES TENTHRÈDES

PARTIE I¹

PAR A. R. GOBEIL²

Sommaire

Le présent travail comprend une étude sur l'action de certains facteurs sur la rupture de la diapause chez les Tenthredes entre autres *Diprion polytomum* Htg. et *Pristiphora erichsoni* Htg. La température optimum d'hibernation serait aux environs de 32° F. Aucune des espèces étudiées n'a pu vivre plus de 10 semaines à 0° F. Le milieu optimum de nymphose pour *Diprion polytomum* serait de 75° F. à 80° F. avec une humidité relative de 85% et plus. Les chocs thermiques au début de l'hibernation provoquent des diapauses plus difficiles à rompre alors que les mêmes chocs à la fin de l'hibernation activent la réactivation. A des températures voisines du point de congélation, les larves peuvent vivre plus de trois semaines complètement submergées dans l'eau. De courtes immersions dans l'eau durant l'hibernation augmentent le pourcentage d'émergence. Le métabolisme est encore plus intense si les immersions se font dans une solution d'acide sulfurique à pH 3. Par contre le glycolle inhibe la réactivation et augmente le taux de mortalité.

Introduction

La diapause chez les insectes présente un intérêt scientifique indéniable, aussi a-t-elle suscité de très nombreux travaux. Par ailleurs, ce problème est fort important au point de vue économique et, en ces dernières années, il est même devenu d'une grande actualité dans l'Est du Canada, à la suite de la découverte en notre pays de la mouche à scie européenne de l'épinette, *Diprion polytomum* Htg. Cet insecte, comme chacun sait, a envahi presque toute notre province et, à date, il a causé des dégâts considérables à nos forêts d'épinette. Or ce qui est le plus intéressant dans le comportement de cette mouche à scie, c'est le fait qu'elle peut avoir deux et même trois générations dans certaines régions, tandis que dans les forêts du Nord, elle n'en a qu'une et, de plus, la pronympe peut rester en diapause dans son cocon six et même sept ans avant de donner naissance à un adulte.

Evidemment, cette longue diapause présente des inconvénients mais aussi des avantages pour la survivance de l'espèce. Il est vrai que des individus subissant un arrêt de plusieurs années dans leur développement auront un potentiel biotique beaucoup plus bas que s'ils avaient deux générations ou plus par année. Il est non moins vrai que dans la partie nord de son habitat, les principaux ennemis de *D. polytomum*, les musareignes et les écureuils, seront des agents de contrôle plus efficaces, la pronympe étant à leur merci durant une plus longue période. D'un autre côté, certaines méthodes de lutte chimique ou sylvicole, de même que certains facteurs naturels de réduction

¹ Manuscrit reçu le 12 mai 1941.

Contribution No 11, Service d'Entomologie, Ministère des Terres et Forêts, de la Chasse et de la Pêche, Québec, Qué.

D'après une thèse soumise à la Faculté des Gradés de l'Université McGill en accomplissement partiel des exigences requises pour le degré de Docteur en Philosophie.

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auront moins de succès parce que n'affectant qu'une partie de la population, l'autre étant au repos dans le sol. Ainsi durant l'été 1940 une maladie infectieuse a réduit la population larvaire de façon substantielle dans la Gaspésie (49). Mais son effet sur la population future de l'insecte sera loin d'être aussi sensible qu'il le fût, par exemple, dans le Vermont et le New Hampshire, alors qu'à certains endroits la mouche à scie fût à peu près éliminée, apparemment par la même maladie (33). Pour toutes ces raisons, une étude des facteurs pouvant affecter la diapause de la mouche à scie européenne de l'épinière est donc très importante.

Pour cela, j'ai d'abord cherché à établir l'influence de différentes températures d'hibernation et de nymphose afin d'en arriver à la température optimum pour chacun des deux états. A ces températures optima et pour différentes périodes d'hibernation, j'ai fait agir, soit au cours de l'hibernation, soit durant la nymphose, soit encore durant ces deux périodes, d'autres facteurs ou agents susceptibles de provoquer la rupture. Puis, à l'aide d'analyses statistiques, j'ai pu évaluer et comparer l'influence de chacun de ces facteurs ou agents dont les principaux furent les suivants: variations brusques de température ou chocs thermiques; atmosphère desséchée et très humide; immersions dans l'eau; immersions dans des solutions acides à des pH comparables à ceux qui existent dans le sol*; immersions dans de l'acide sulfurique concentré*.

Remerciements

Les expériences préliminaires à ce travail furent exécutées au Collège Macdonald au cours de l'hiver 1938-1939 sous la direction du Prof. Melville Du Porte, dont les conseils me furent alors très utiles. Il me fait plaisir de lui en exprimer ici toute ma gratitude.

Je suis aussi très reconnaissant au Prof. Samuel Graham de l'Université de Michigan pour avoir bien voulu me diriger dans la préparation de mon programme pour l'hiver 1940-1941 et m'avoir fait de précieuses suggestions.

Enfin je tiens à remercier le Prof. R. Summerby du Collège Macdonald et le Dr J. W. Hopkins du Conseil National de Recherches pour l'aide qu'ils m'ont donné lors de l'arrangement de mes expériences et de l'analyse statistique de mes résultats.

Historique

Le terme diapause fut employé pour la première fois par Wheeler pour désigner une période de repos durant le développement embryonnaire. Plus tard, Henneguy a étendu le sens de ce mot à tout arrêt dans le développement ontogénétique d'un animal, depuis la fécondation de l'œuf jusqu'à l'âge adulte. Nous aurions donc ainsi des diapauses embryonnaires, larvaires, et nymphales.

Les mots hibernation et estivation sont aussi employés pour signifier les diapauses survenant durant l'hiver et l'été. Shelford (106) et Bodine (18), cependant, font une distinction entre la diapause et l'hibernation. Il faudrait

* Ceux-ci seront examinés dans la Partie II de cet article.

appeler hibernation les cas où l'arrêt dans le développement est causé par la température seulement (Bodine) ou par des conditions défavorables (Shelford). La diapause, d'un autre côté serait inhérente à l'espèce (Bodine) et le développement arrêterait spontanément (Shelford). Ce serait là une distinction très importante s'il était prouvé que la diapause est un phénomène héréditaire ou spontané, mais plusieurs auteurs ne partageant pas cette manière de voir.

La diapause est un sujet qui a été très discuté et sur lequel des opinions variées ont été émises. Athanasiu (2) en 1909 fit une revue très complète des recherches exécutées jusqu'au début de notre siècle sur ce qu'on appelait alors l'hibernation ou l'estivation. Plus récemment, Cousin (30) dans son étude sur le même sujet a résumé de façon substantielle les travaux publiés sur la diapause antérieurement à 1930. C'est pourquoi, je ne donnerai ici qu'un aperçu des plus importantes et des plus récentes théories. Pour plus ample information, on consultera avec avantage les auteurs ci-dessus mentionnés.

Les principaux facteurs et organismes qui ont été mis en cause pour expliquer la diapause chez les insectes peuvent être classés comme suit: *a.* les glandes hormonogènes; *b.* l'hérédité; *c.* les facteurs internes; *d.* les facteurs physiques ou externes.

Wigglesworth (120-121) a beaucoup étudié l'influence des hormones sur le développement des insectes. Après avoir opéré des nymphes de *Rhodnius prolixus* et leur avoir enlevé l'hormone provoquant les mues, le "corpus allatum" situé dans la tête, il a montré que les sujets ainsi traités ne croissaient pas du tout, même après avoir absorbé un repas complet. De tels individus sont en diapause; et la croissance ou la métamorphose n'aura lieu que si l'hormone requise est introduite dans le sang. Ceci dit-il suggère naturellement que la diapause chez les autres insectes, *quand elle n'est pas occasionnée par les effets directs du milieu*, peut résulter d'un affaiblissement temporaire de l'hormone promouvant la croissance.

Parmi les auteurs considérant la diapause comme héréditaire ou rythmique, mentionnons simplement: Babcock (3, 4), Baumberger (10, 11), Bodine (15-21), Holmquist (53, 54), Pictet (77-82), et Readio (86). Tous admettent, cependant que cette qualité inhérente peut être modifiée à un plus ou moins grand degré par les facteurs du milieu.

Parker et Thompson (68) ont associé la diapause de la pyrale du maïs avec le développement des organes reproducteurs. Au printemps, certains changements définis se produisent chez les rudiments d'organes génitaux. Ils ont constaté quatre phases de développement des gonades mâles, et la deuxième phase serait le début d'une activité physiologique qui, suivant le cas, élimine ou met fin à la diapause. Cependant, comme l'a fait remarquer Cousin, cet état particulier des organes génitaux peut caractériser la diapause de *Pyrausta nubilalis*, mais n'en est pas le facteur déterminant et les causes doivent être cherchées ailleurs. Les mêmes remarques s'appliquent au processus enzymatique étudié par Townsend (114) durant l'hibernation de la pyrale du pommier *Carpocapsa pomonella*, et le peu de catalase noté par Spooner (109)

chez le même insecte. Tous ces changements, tout comme la réduction dans les échanges respiratoires (74-75), la perte d'eau (101), et de gaz carbonique (116), sont des modifications prenant place durant l'hibernation et caractérisant la diapause, mais, apparemment, n'en sont pas les causes déterminantes.

Nombreux sont ceux qui ont attribué la diapause à une intoxication de l'organisme. Suivant les uns, tels, Baumberger (11), Bellion (13), Child (27), Dubois (35-37), Kamenskii et Paikin (57), cette intoxication serait le résultat d'accumulation de gaz carbonique dans l'organisme, alors que pour d'autres (91-97), elle serait liée à une condition de surcharge de produit d'excrétion. Plus récemment, Roubaud (98) a attribué l'hibernation obligatoire de la mouche *Phlebotomus papatasi* à l'influence exercée par l'organisme maternel. Lorsque les ovules sont retenus dans les ovaires pendant un temps anormal, ils s'y surchargent d'éléments inhibiteurs du développement. Les effets de ce blocage se feront sentir ultérieurement amenant la diapause. D'après lui il s'agit bien là "d'un effet de surcharge métabolique, puisque le forçage à la chaleur entraîne habituellement la mort. C'est la détente prolongée, assurée par le repos de l'hibernation, qui permet aux individus asthéniques de ranimer leur activité." L'hypothèse de Bodine (15-21) se rapproche beaucoup de celle de Roubaud. La plupart de ses expériences ont eu pour sujets des sauterelles. Pour expliquer la diapause des œufs de *Melanoplus*, il introduit l'expression "diapause factors" ou facteurs de diapause, qui peuvent être soit chimiques, physico-chimiques ou encore des facteurs physiologiques-génétiques, présents dans l'œuf en quantité plus ou moins constante lors de l'oviposition. Suivant Bodine, ces facteurs "x" sont extrêmement sensibles aux basses températures, au-dessous du zéro ou seuil de développement, et peuvent être complètement détruits ou supprimés.

Il n'y a probablement pas de facteur qui ait été plus étudié que la température. C'est pourquoi, pour éviter toute longueur, je me contenterai de mentionner ici les travaux d'Abeloos (1), Ball (9), Balachowsky (6), Brumpt (23), Sanderson (103), et Volkonsky (118). Quant à l'humidité, la majorité des auteurs reconnaissent que ce facteur joue un rôle important avec la reprise des activités après l'hibernation. D'autres expérimentateurs vont plus loin et croient que pour certains insectes l'humidité est le facteur de premier ordre sinon le seul concerné avec la diapause.

Des résultats très intéressants furent obtenus par Sabrosky et ses collaborateurs (102) en soumettant les œufs de la sauterelle, *Acridium arenosum angustum* à l'influence de radiations électriques blanches et violettes. Normalement cette sauterelle hiverne à l'état d'œuf, mais au moyen de ces irradiations, ils ont pu obtenir une génération d'hiver et briser la diapause, alors que la chose était impossible en soumettant simplement les œufs aux températures élevées de la serre. Par ailleurs, Hufnagel et Nabias (55) ont montré que l'action du radium n'apporte aucune modification dans le développement des *Calligrapha* et *Hyponometa*, et Ray (85) a constaté que des irradiations de $\frac{1}{2}$ à 4 heures retardaient le développement de *Melanoplus differentialis*

et pouvaient même avoir un effet mortel. Ceci concorde avec les expériences de Hussey, Thompson, et Calhoun (56) qui ont trouvé que chez les larves de *Drosophila*, la durée moyenne de la période prénymphale est une fonction croissante de la durée d'irradiation aux rayons X.

Dans ce qui est probablement le travail le plus approfondi et le plus complet sur ce sujet, Cousin (30) soutient que la diapause est le résultat d'une perturbation physiologique spécifique en liaison directe ou indirecte avec le séjour de l'insecte dans des milieux défavorables.

Ses expériences avec *Lucilia sericata*, démontrent que les conditions brisant la diapause sont généralement liées très étroitement avec celles qui l'ont déterminée. Ainsi une diapause causée par une sécheresse peut être rompue par un séjour dans un milieu où l'air est saturé de vapeur d'eau. Mais lorsque la durée de la diapause a été assez longue ou que les facteurs qui l'ont provoquée ont agi de façon très intense, cette relation entre la cause de la période de repos et la reprise du développement n'est pas aussi évidente. Pour obtenir la rupture de la diapause, il peut être alors nécessaire d'adjoindre au facteur qui l'a occasionnée, d'autres provoquant certains chocs et jouant le rôle d'excitant.

Matériel et Méthodes

PROVENANCE DES INDIVIDUS

Pour mes études préliminaires, saison 1938-1939, je me suis servi de cocons provenant des échantillons reçus au cours de l'été en rapport avec notre inventaire relatif aux insectes forestiers (48). Nous avions des spécimens nous venant d'à peu près tous les comtés de la Province et pour chaque individu une fiche donnant entre autres détails: son identité, sa plante nourricière, et la date de formation du cocon. J'espérais ainsi pouvoir montrer l'action du climat, de l'alimentation, et des conditions saisonnières sur la durée de la diapause, mais ce me fut impossible; les renseignements que je possédais étant de nature trop générale. De plus, mes sujets venant de différentes localités et ayant passé une partie de leur vie dans leur milieu naturel, certains facteurs particuliers sur lesquels je ne savais rien du tout auraient fort bien pu être la cause première de ces long sommeils. C'est la seule façon d'expliquer certaines réactions apparemment contradictoires obtenues en 1938-1939.

Par ailleurs l'hétérogénéité des échantillons constituant mes lots a quelquefois invalidé mes résultats et, à moins qu'elle ne fût très significative, il n'était pas toujours possible d'attribuer aux traitements la différence de développement observée entre deux lots. En effet, il y avait d'autres causes de variations, régionales, alimentaires, ou peut-être même génétiques qui n'avaient pas été éliminées et qui pouvaient avoir sur les sujets une plus grande influence que les traitements expérimentés. Il m'est arrivé par exemple d'avoir des pourcentages d'émergence très différents pour des lots placés dans des conditions presque identiques. Sur examen des échantillons formant ces lots, j'ai constaté que plusieurs individus, de volume inférieur à la moyenne, fai-

saient partie d'échantillons contenant un nombre considérable de larves élevées dans des bocalux trop petits, d'où insuffisance de nourriture. Ces larves étaient évidemment moins résistantes que les larves normales du deuxième lot, de là un plus fort pourcentage de mortalité et moins d'émergence.

En 1940, j'ai réduit les causes incontrôlables de variations en me procurant un stock aussi homogène que possible. Tous les cocons de *D. polytomum* (50,000) furent recueillis à la même date, le 26 août et au même endroit, dans un peuplement mêlé (épinette, sapin, et bouleau), à Ste-Florence, comté de Matapédia. Quant aux 25,000 cocons de *P. erichsoni*, ils furent ramassés entre le 26 août et le 1er septembre dans le canton Thorne, comté de Pontiac.

ESPÈCES ÉTUDIÉES

Diprion polytomum Htg.

Pour une meilleure compréhension des expériences qui vont suivre il me paraît indispensable de dire quelques mots sur le cycle de *D. polytomum*. Auparavant, je dois d'abord attirer l'attention sur la classification de Benson (14) qui inclut *D. polytomum* dans son nouveau genre *Gilpinia* séparé de *Diprion* surtout par le développement des "cenchri" du meso- et metascutellum. Les caractères invoqués par Benson justifient peut-être l'érection de ce nouveau genre, mais comme l'usage de ce nom est encore plutôt restreint, j'ai cru préférable d'utiliser ici *Diprion polytomum*, nom sous lequel l'espèce est maintenant connue de tous les forestiers de Québec.

Cette Tenthrede apparemment introduite d'Europe et trouvée dans la Gaspésie à l'état épidémique en 1930 (8) ne s'attaque qu'à l'épinette. L'insecte hiverne dans la mousse ou l'humus à l'état larvaire dans un cocon brun. Au début de l'été, un pourcentage plus ou moins grand des individus se transforment d'abord en pronympe puis en nymphe et finalement en adulte dont la plupart sont des femelles. En effet, les mâles, sont très rares et la reproduction est parthénogénésique. L'adulte est plutôt indolent et bien qu'il puisse voler sur de longues distances, il ne prendra son vol que par des journées ensoleillées, lorsque la température est relativement élevée. Les oeufs sont pondus à raison de un par feuille sur le vieux feuillage. L'éclosion de la larve se produit de 8 à 12 jours plus tard; elle se nourrit de préférence sur le vieux feuillage ne s'attaquant à la nouvelle pousse que plus tard dans la saison, et seulement lorsqu'il ne lui reste rien d'autre à sa portée. Au cours de sa vie larvaire l'insecte mue cinq fois et passe par six stades, alors qu'ayant atteint pleine maturité, il se laisse choir de l'arbre pour tisser son cocon dans la mousse. Entre le sixième stade larvaire, qui ne dure que quelques jours, et la pronympe, certains auteurs reconnaissent un autre stade, celui d'éonympe, forme sous laquelle l'insecte hivernerait. Dans ce travail, l'éonympe est tout simplement désignée par le mot larve. L'emploi de ce terme ne peut prêter à confusion puisque cette étude ne porte que sur le développement à l'intérieur du cocon comprenant trois stades, à savoir: la larve ou éonympe, la pronympe et la nymphe.

Dans le nord de la province il n'y a qu'une génération par année alors que dans le district de Montréal et des Cantons de l'Est on peut avoir deux générations et trois dans l'est des Etats-Unis.

Dans la Gaspésie des adultes sont éclos de cocons en diapause depuis six ans, la durée de cette léthargie variant suivant les régions et les années. D'après Balch (7) le pourcentage moyen d'émergence par année approcherait 20% dans la Gaspésie alors que dans le centre du Nouveau-Brunswick il serait d'environ 70%. Pour une même localité, ce pourcentage varie beaucoup d'une année à l'autre; dans le comté de Kamouraska seulement 2% des larves hibernantes ont donné naissance à des adultes à l'été 1934. Au même endroit, pour les trois années qui suivirent, le pourcentage d'émergence a varié de 12 à 28%. Suivant Balch, la température et l'humidité seraient des facteurs de première importance pour la rupture de la diapause. Un séjour d'environ trois mois à des températures au-dessous du seuil de développement serait nécessaire pour obtenir le maximum d'émergence. Ce maximum serait de plus influencé par le degré d'humidité et le contact de l'eau. Sous élevages contrôlés, Balch a obtenu pour 21 générations consécutives une lignée sans diapause. D'un autre côté, les descendants de femelles provenant de la Gaspésie montrent une forte tendance pour l'hibernation et, à l'exception d'un cas, il a été impossible de l'éliminer après la première ou la deuxième génération. Tout en admettant l'importance de la température et de l'humidité, Balch croit donc que chez *D. polytomum* la diapause est héréditaire et les variations chez différentes lignées ou races seraient de nature génétique.

Pristiphora erichsoni Htg.

Les insectes hibernant dans le sol ont généralement surabondance d'humidité. L'habitat du cocon de la mouche à scie de l'épinette, bien que souvent saturé, est, cependant, relativement sec, surtout lorsque la larve s'est développée sur l'épinette blanche dans des peuplements mêlés où le sol est généralement bien drainé. Par contre, le cocon de la mouche à scie du mélèze étant dans un milieu presque toujours saturé d'eau et souvent même en contact avec l'eau, cette espèce est bien représentative des Tenthredes hibernant en milieu excessivement humide. Soumis à l'action des mêmes agents, cet insecte devrait donc dans maints cas réagir différemment de *D. polytomum*. C'est pourquoi, en 1940, la plupart des expériences faites sur *Diprion*, furent répétées avec *Pristiphora erichsoni*.

Probablement indigène au pays, la mouche à scie du mélèze fut constatée en Amérique pour la première fois dans l'Etat du Massachusetts en 1881 par Sargent (cf. 52). Depuis, la mouche à scie du mélèze s'est révélée l'insecte le plus nuisible du mélèze. Son cycle vital ne diffère pas sensiblement de celui de *Diprion*. La larve hiverne aussi dans son cocon sous la mousse. Ici encore les mâles sont peu fréquents et la reproduction est partiellement parthénogénésique. Les oeufs sont pondus en rangées dans des fentes taillées sur les pousses terminales et les larves se nourrissent exclusivement des feuilles du mélèze.

Il n'y a dans la littérature que très peu de détails sur la durée de la diapause de la mouche à scie du mélèze. Parmi les nombreuses publications consultées, je n'en ai trouvé qu'une seule (5) où il en soit fait mention. Lors de ses études sur le contrôle naturel de *Pristiphora erichsoni* dans le Nouveau-Brunswick, cet auteur remarqua qu'en 1921 environ 25% des larves étaient encore dans leur cocon après un an d'hibernation. Depuis, plusieurs années de manipulation de cocons de cette espèce pour la propagation de parasites ont permis à M. Baird (lettre du 12 novembre 1940) de constater que d'une année à l'autre et pour une même localité, il y a des variations considérables dans la durée de l'hibernation. Généralement, cependant, un très petit pourcentage des larves, moins de 1%, demeurent en léthargie pour plus d'un hiver, bien qu'il soit arrivé que ce pourcentage atteigne 25% et que quelques larves fussent encore vivantes dans leur cocon après deux ans.

Les observations faites par J. J. DeGryse (lettre du 24 octobre 1940) à Indian Head de 1923 à 1925, bien que de nature très générale, indiqueraient que dans l'Ouest du Canada la diapause peut durer deux et même trois ans. Des diapauses prolongées furent aussi observées il y a déjà plusieurs années dans l'Itasca Park, Minnesota (S. Graham, lettre du 21 avril 1941).

D'après mes propres expériences 1940-1941, il n'y a pas de doute que certaines années, dans certaines localités, la plus grande partie des larves subissent des diapauses qu'il est très difficile de rompre. Il est vrai que les traitements que j'ai fait subir aux individus de cette espèce causèrent dans plusieurs cas 100% de mortalité. Il n'en reste pas moins, cependant, qu'à la fin de mes expériences j'avais encore plus d'un millier de sujets vivants et sur ce nombre j'en ai eu moins d'une dizaine d'adultes. Pourtant les mêmes traitements en 1938-1939 avaient provoqué jusqu'à 40% d'éclosion. Je ne puis expliquer cette différence de comportement qu'en l'attribuant aux conditions de milieu dans lesquelles vécurent ces individus avant leur arrivée au laboratoire, conditions ayant provoqué des diapauses tellement durables qu'il me fut, dans la majorité des cas, impossible de les briser au moyen des traitements employés.

Autres Espèces

Alors que les études de 1940 portèrent uniquement sur *Diprion polytomum* et *Pristiphora erichsoni*, quelques observations supplémentaires furent faites en 1938-1939 sur les Tenthredines suivantes en plus, bien entendu, des deux espèces ci-dessus mentionnées qui ont toujours constitué la plus grande partie du matériel.

Espèces	Nombre de spécimens
<i>Pikonema alaskensis</i>	662
<i>Pikonema dimmockii</i>	234
<i>Neodiprion lecontei</i>	319
<i>Neodiprion pinetum</i>	108
<i>Hylotoma pectoralis</i>	273

TECHNIQUE

Saison 1938-1939

À l'automne de 1938, nous avons un total de 13,500 cocons obtenus grâce à notre enquête sur les insectes forestiers. Tous ces cocons furent répartis en 24 lots comprenant chacun 350 à 600 individus. Nous avons pris soin d'inclure dans chaque lot des échantillons de 1 à 10 cocons provenant de diverses parties de la Province et gardés séparément dans des fioles de 15 × 45 mm. bouchées avec de l'ouate. Le 1er octobre tout le matériel fut expédié dans un entrepôt frigorifique et remis pour des périodes variant de 4 à 14 semaines dans des chambres à températures constantes soit: 32° F., 15° F., et 0° F., respectivement. Les abaissements de température furent graduels, c'est-à-dire qu'avant leur séjour à 0° F., par exemple, les individus passèrent d'abord trois jours à 45° F. puis trois jours à 32° F. et à 15° F.

De l'entrepôt frigorifique, les cocons furent expédiés au Collège Macdonald et amenés progressivement, par bonds de 15° F. (trois jours d'exposition à chaque échelon), aux températures de 60° F., 75° F., 80° F., 85° F., et 90° F., dans une atmosphère très sèche soit: 20 à 22% d'humidité relative dans les chambres de 85° F. et 90° F., environ 25% pour celles de 75° F. et 80° F., et 35% dans la chambre de 60° F. Il est à remarquer qu'au cours du trajet de Québec à Ste-Anne de Bellevue (une journée) il me fut impossible de contrôler la température. On m'a assuré, cependant, qu'elle oscillait entre 34° F. et 45° F.

À leur arrivée au Macdonald, chaque lot fut subdivisé en deux ou trois sous-lots de 150 à 200 cocons et soumis aux températures de nymphose ci-haut mentionnées. Auparavant, cependant, un échantillon de 50 cocons par lot fut prélevé et analysé afin de déterminer le taux de mortalité au cours de l'hibernation, ce taux étant ensuite appliqué aux sous-lots constituant le lot original.

Finalement, après un séjour de deux mois aux températures de nymphose, tous les cocons furent ouverts, et le taux de mortalité et de développement en incubateur fut calculé. Les chiffres obtenus furent alors utilisés pour estimer l'action de différents milieux sur les individus.

Saison 1940-1941

La technique employée et les appareils utilisés en 1938 furent grandement améliorés en 1940. Tout d'abord nous avons cherché à obtenir un stock de cocons aussi homogène que possible en prélevant nos échantillons au même endroit et vers la même date. Cette façon de procéder a réduit au minimum les variations dues à l'origine des individus. À leur arrivée à Québec, les cocons de *D. polytomum* furent répartis en lots de 125 cocons et ceux de *P. erichsoni* en lots de 110 cocons, placés dans des tubes de verre de 22 × 95 mm., dans lesquels l'aération fut assurée en bouchant les extrémités avec de la mousseline.

Dès le 1er septembre, tous les cocons furent placés à basses températures dans notre réfrigérateur. Cet appareil comprend trois chambres tout à

fait indépendantes et qui peuvent être réglées à n'importe quelle température entre 0° F. et 45° F. au moyen de thermostats De Khotinsky. Dans nos expériences nous avons utilisé trois températures, soit 15° F., 34° F., et 45° F., mais la plupart des individus hibernèrent à 34° F. A la fin de l'hibernation, le taux de mortalité fut calculé en prélevant 25 cocons de chaque lot de *D. polytomum* et 10 de chaque lot de *P. erichsoni*. En conséquence il restait exactement 100 individus par lot lorsque les cocons furent transférés à l'incubateur. Le nombre d'individus examinés dans chaque lot afin de déterminer le taux de mortalité durant l'hibernation peut paraître plutôt faible, mais il est au contraire suffisamment élevé parce qu'au cours de l'hibernation, de 4 à 10 lots reçurent le même traitement.

Les expositions aux hautes températures eurent lieu dans trois incubateurs de 6 × 3 × 3 pieds à double parois de "masonite". La chaleur étant fournie par une plaque chauffante, contrôlée par un thermostat De Khotinsky, il n'y avait donc dans les incubateurs aucune source lumineuse. L'humidité désirée fut maintenue au moyen de l'appareil décrit par Fulton (43) et contrôlée par un humidistat "Fries". Incidemment, cet appareil de Fulton est peu dispendieux et il fonctionne très bien dans nos incubateurs. Pour la ventilation nous employons un éventail, à mouvement oscillatoire, dont la vitesse est réglée par un rhéostat. Afin d'assurer une bonne ventilation dans chaque lot avec le moins de perte d'espace possible, les incubateurs, de même que le réfrigérateur, sont équipés de chevalets de 1.5 × 2 × 2 pieds en tôle galvanisée avec sur les côtés des encoches à tous les trois pouces, sur lesquelles embarquent les treillis métalliques supportant les tubes de verre contenant les cocons. Les incubateurs furent réglés de façon à obtenir les températures et les humidités suivantes: Incubateur A, 75° F., 56% R.H.*; Incubateur B, 85° F., 85% R.H.; Incubateur C, 75° F., 85% R.H. D'après nos records de thermographes et hygrographes, les premiers vérifiés trois fois par jour sur des thermomètres Taylor, nous avons pu contrôler la température à 1° F. près et l'humidité relative à 3%.

Comme en 1938, toutes les expériences furent discontinuées après 60 jours d'incubation. Les cocons furent alors ouverts et les individus classés dans une des quatre catégories suivantes: parasités, morts, éclos, et vivants; les individus morts et vivants étaient de plus subdivisés en: larves, pupes, et adultes. Finalement les pourcentages de mortalité et d'émergence furent calculés comme suit:

$$\text{Pourcentage de mortalité} = \frac{100 (\text{nombre de cocons morts})}{(\text{nombre total de cocons}) - (\text{cocons parasités})}$$

$$\text{Pourcentage d'émergence} = \frac{100 (\text{éclos} + \text{pupes et adultes vivants après 60 jours d'incubation})}{(\text{nombre total de cocons}) - (\text{cocons parasités et morts})}$$

Les mots développement et émergence tels qu'employés dans ce travail ne sont pas tout à fait appropriés. En effet pour calculer le pourcentage de développement réel, si l'on considère la rupture de la diapause seulement, il

* R.H. = Humidité relative.

aurait peut-être été préférable d'ajouter au numérateur de la formule les pupes et adultes morts. Mais ce qui m'intéressait tout d'abord, ce n'était pas tant de démontrer l'influence de tel ou tel facteur sur la rupture de la diapause, comme son influence sur le pourcentage d'éclosion, car au point de vue économique c'est ce qui compte le plus. En outre le mot émergence n'est pas non plus très exact pour désigner le pourcentage d'émergence tel que calculé, puisqu'avec les cocons éclos sont inclus ceux contenant des pupes et adultes vivants. Il est fort probable, en effet, que si les expériences avaient été prolongées de quelques jours, l'éclosion aurait eu lieu.

Enfin, pour le calcul du pourcentage d'émergence, non seulement les cocons parasités, mais aussi les cocons morts furent éliminés, car dans la plupart des cas la majorité de ces individus étaient morts avant le commencement de l'expérience. Il est arrivé que les traitements subis par certains lots ont occasionné une mortalité plus élevée que la moyenne, mais ces cas furent mis à jour par les analyses de variance et furent pris en considération lors de la comparaison des différents traitements.

Influence de la température et de l'état hygrométrique de l'atmosphère

ACTION DES TEMPÉRATURES D'HIBERNATION

En 1938-1939, trois températures d'hibernation furent mises à l'essai: 32° F., 15° F., et 0° F. En 1940-1941, bien que tous les lots de *Pristiphora erichsoni*, ainsi que la plupart de ceux de *Diprion polytomum* hibernèrent à 34° F., plusieurs expériences furent effectuées à 45° F. et 15° F.

L'effet de la température d'hibernation a été considéré sous quatre aspects: 1. Mortalité durant l'hibernation; 2. Limite vitale inférieure; 3. Mortalité durant la nymphose; 4. Action sur l'émergence.

Mortalité durant l'hibernation

Comme le font soupçonner les résultats présentés au Tableau I et comme le prouve d'ailleurs l'analyse statistique des mêmes résultats (Tableau II), la valeur de "F" obtenue pour les variations dues aux traitements 1.42 nous donne une probabilité supérieure à 5% parce que plus petite que la valeur de F qui, avec une probabilité de 5%, est de 1.97 lorsqu'on a 11 et 55 degrés

TABLEAU I

Diprion polytomum, POURCENTAGE DE MORTALITÉ DURANT L'HIBERNATION

Température d'hibernation	Durée de l'hibernation en jours			
	42	56	84	112
	Mortalité			
45° F.	20.0	25.3	21.3	20.0
34° F.	17.3	20.7	28.7	19.3
15° F.	17.3	28.7	20.7	22.0

TABLEAU II
ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT
L'HIBERNATION
(Voir Tableau I)

Sources de variation	Degrés libres (D.L.)	Variance
Répétitions	5	157.16*
Traitements	11	88.40
Erreur	55	62.22
Total	71	Erreur standard = 7.89

* Dépasse "erreur" variance, niveau significatif de 5%.

libres (108). Si l'on considère, comme je l'ai fait partout dans mes analyses, que les traitements ou facteurs étudiés sont responsables des différences observées seulement lorsque celles-ci ont une probabilité inférieure à 5%, alors les différences constatées dans la moyenne de mortalité aux trois températures d'hibernation éprouvées en 1940 seraient simplement l'effet de fluctuations dues à l'échantillonnage. De plus la mortalité n'est pas influencée par la durée du séjour à 45° F., 34° F., ou 15° F., que ce séjour soit de 42 ou de 116 jours.

Le taux moyen de mortalité enregistré à la fin de l'hibernation pour les 72 lots exposés aux températures ci-haut mentionnées, fut de 22.8%. Ceci ne veut pas dire, toutefois, qu'un tel pourcentage d'individus aient succombé au cours de l'hibernation, car plusieurs étaient déjà morts au début des expériences et toutes ces moyennes ne peuvent donc servir qu'à comparer différents traitements et à estimer la mortalité en incubateur.

Limite vitale inférieure

Aucune des espèces étudiées en 1938 n'a pu résister très longtemps à une température de 0° F. Dans mes expériences, en effet, 38% des larves de *D. polytomum* étaient mortes après un stage de sept semaines à 0° F. Une semaine plus tard le taux de mortalité atteignit 65% et, après 10 semaines à 0° F., il ne restait plus qu'une seule larve vivante sur un total de 190 cocons. Dans ce dernier cas, environ 50% des individus avaient conservé l'apparence de larves vivantes, mais leur corps était flasque et si on les pressait légèrement elles ne reprenaient pas leur forme normale comme c'est le cas pour les larves en diapause seulement. Le reste des sujets avaient déjà pris une teinte jaunâtre et la tête était d'un vert foncé.

Dans un lot contenant 50 cocons de *Pristiphora erichsoni* et 45 d'*Hylotoma pectoralis*, il ne restait plus une seule larve vivante après un stage de 10 semaines à 0° F. Après 14 semaines à la même température, l'examen d'un lot constitué de 170 cocons de *Neodiprion pinetum* décelait 100% de mortalité.

Chez les Tenthredes, la limite vitale inférieure, lorsque les larves sont en diapause dans le cocon, nous paraît être entre 15° F. et 0° F.

Mortalité durant la nymphose

A la fin de nos expériences après 60 jours en incubateur, la moyenne de mortalité aux trois températures d'hibernation fut, comme suit (Tableau III): à 45° F., 43.5%; à 34° F., 47%; à 15° F., 47.9%. Comme on le voit, les températures d'hibernation par elles-mêmes n'affectent en rien le pourcentage de mortalité subséquente en incubateur.

TABLEAU III

Diprion polytomum, INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE DE MORTALITÉ EN INCUBATEUR

Hibernation		Temp.	R.H.	Temp.	R.H.	Temp.	R.H.
Température	Durée en jours	85°	85%	75°	85%	75°	56%
		Mortalité					
45° F.	42	48.9		40.0		55.6	
	56	37.9		47.9		28.8	
	84	47.8		34.0		45.7	
	112	35.2		43.1		47.1	
34° F.	42	66.5		27.0		67.3	
	56	25.0		32.2		36.9	
	84	47.3		49.9		68.9	
	112	45.1		45.6		70.8	
15° F.	42	36.3		35.8		83.9	
	56	26.0		39.6		48.3	
	84	37.5		51.4		40.7	
	112	60.7		52.7		61.4	

Par ailleurs, la résistance de *Diprion polytomum* aux différentes températures et humidités de nymphose est influencée par le degré de froid où s'est effectuée l'hibernation. En effet, au Tableau IV, la variance de l'interaction de la température d'hibernation sur les conditions de nymphose est 5.28 fois plus grande que la variance de l'erreur expérimentale, laquelle est associée aux variations dues au hasard et désignée par plusieurs, simplement par le mot "erreur". L'erreur standard de la moyenne pour cette interaction est égale à:

$$\frac{E.S.}{\sqrt{N}} = \frac{9.20}{\sqrt{8}} = 3.253$$

L'erreur standard de la différence entre deux moyennes étant $3.253 \sqrt{2} = 4.60$, et la valeur de "t" pour $p = 5\%$ avec $n = 35$, étant 2.03, alors pour que la différence entre deux moyennes de mortalité à différentes températures d'hibernation et dans différents milieux de nymphose soit bien significative elle devra donc être supérieure à $4.60 \times 2.03 = 9.34$.

A 85° F., et 85% R.H., le pourcentage de mortalité n'est pas affecté par la température d'hibernation: la moyenne minimum étant de 40.2% à 15° F.

TABLEAU IV
ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE
 (Voir Tableau III)

Sources de variation	D.L.	Variance
Blocs	1	0.1
Traitements	35	398.35**
Temp.	2	127.15
Jours	3	953.50**
Nymphose	2	1391.68**
Temp. × jours	6	165.54
Temp. × nymph.	4	432.62**
Jours × nymph.	6	403.39**
Temp. × jours × nymph.	12	241.67**
Erreur	35	84.2
Total	71	Erreur standard = 9.20

** Dépasse "erreur" variance, niveau significatif de 1%.

et la moyenne maximum 46.2% lorsque l'hibernation a lieu à 34° F. Il n'en est pas de même à 75° F. et 85% R.H., alors que la moyenne de mortalité n'est que de 33.7% à 34° F. d'hibernation et 44.9% à 15° F. Comme question de fait, lorsque l'on considère seulement les facteurs température et humidité, l'hibernation à 34° F., et la nymphose à 75° F. et 85% R.H. sont la combinaison donnant la mortalité minimum et l'émergence maximum. Incidemment, c'est ce que faisait prévoir mes résultats en 1938 et c'est pourquoi en 1940 la plus grande partie des expériences furent accomplies dans ces conditions de milieu.

Lorsque la nymphose se fait à 75° F., 56% R.H., la mortalité est beaucoup plus élevée si l'hibernation s'effectue à des températures voisines ou inférieures au point de congélation que si elle se produit à des températures bien supérieures à ce point. Ainsi, la moyenne de mortalité à 34° F., et 15° F., s'éleva à 61 et 58.6% respectivement, alors qu'elle ne fut que 44.3% à 45° F., ce qui est nettement significatif. *Diprion polytomum* est donc beaucoup plus sensible aux basses températures d'hibernation dans un milieu sec que dans un milieu humide.

Action de la température d'hibernation sur l'émergence

Chez *Diprion polytomum*, il n'y a pas de différence marquée dans le pourcentage de développement après une hibernation à 45° F. et 34° F. A 45° F., la moyenne d'émergence pour 800 individus fut de 10.7%, et à 34° F., pour le même nombre de cocons, j'obtins 9.8% d'émergence; cette différence est tout à fait négligeable comme le démontre l'analyse de variance du Tableau VI.

En 1940-1941, il me fut absolument impossible de provoquer la rupture de la diapause au moyen des températures et des humidités relatives ordinaires de nymphose après une hibernation à 15° F. Cependant, au cours de la saison

TABLEAU V

Diprion polytomum, INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE D'ÉMERGENCE

Hibernation		Températures et humidités relatives durant la nymphose			
Température	Durée en jours	Temp.	R.H.	Temp.	R.H.
		75°	85%	75°	56%
		Émergence			
45° F.	84	12.3		14.4	
	112	9.8		6.4	
34° F.	84	10.6		7.4	
	112	9.6		11.3	

TABLEAU VI

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau V)

Source de variation	D.L.	Variance
Blocs	1	10.8
Traitements	7	13.10
Erreur	7	16.84
Total	15	Erreur standard = 4.104

1938-1939, sur un total de 1200 individus, la moyenne générale de développement après hibernation à 15° F. atteint 12.7% avec un maximum de 22.7% pour un lot de 200 cocons à 75° F., 22% R.H. Ces résultats apparemment contradictoires au cours des deux années sont assez difficiles à expliquer. Il se peut que ce soit dû aux conditions différentes dans lesquelles s'est effectué le développement larvaire.

DÉTERMINATION DE LA TEMPÉRATURE ET DE L'HUMIDITÉ OPTIMA DE NYMPHOSE

Au cours de mes études préliminaires en 1938, les températures de nymphose mises à l'essai varièrent de 60° F. à 90° F., associées à une atmosphère toujours très sèche soit : 35% R.H. à 60° F., et 20 à 25% pour les températures de 75° F. et au-dessus. En 1940-1941, deux températures de nymphose, 75° F. et 85° F., de même que deux humidités relatives, 85 et 56% furent expérimentées.

Développement à 60° F., dans un milieu très sec

A 60° F., il y a reprise de développement, mais il va sans dire que le pourcentage est bien inférieur à celui que l'on obtient à des températures plus

élevées. Après une moyenne de 98 à 112 jours d'hibernation à 32° F., j'eus en 1939, pour *Diprion polytomum*, 8.5% de développement et 6.1% d'émergence, Tableau VII. A cette basse température, le pourcentage d'individus mourant dans le cocon à l'état de pupe ou d'adulte n'est donc que d'environ 2.4%, alors qu'à 75° F. et au-dessus il atteint 25 à 30%. Néanmoins, il est probable que le manque d'humidité (35% dans le premier cas et seulement 22% dans le dernier) fut en partie responsable de cette différence dans la mortalité.

TABLEAU VII

INFLUENCE DE LA TEMPÉRATURE ET DE L'HUMIDITÉ SUR LE POURCENTAGE DE DÉVELOPPEMENT ET D'ÉMERGENCE

(saison 1938-1939)

Hibernation		Température et humidité de nymphose					
		60° F., 35% R.H.		75° F., 22% R.H.		80° F., 22% R.H.	
Température	Jours	Dével.	Emerg.	Dével.	Emerg.	Dével.	Emerg.
32° F.	84	2.5	2.0	10.5	0.5	21.0	9.5
	98	5.7	4.3	41.5	17.0	59.1	11.0
	112	8.7	6.0	31.9	12.8	71.1	31.2
Moyenne		5.6	4.1	28.0	10.1	50.4	17.2
15° F.	84	*		14.3	6.5	3.2	2.2
	98			9.5	4.1	8.5	4.3
	112			22.7	13.4	18.1	3.2
Moyenne				15.5	8.0	9.9	3.2

* Aucune expérience à 65° F., 35% R.H. après une hibernation à 15° F.

Chez toutes les Tenthredes étudiées, après un stage aux températures d'hibernation, il y a reprise de développement à des températures aussi basses que 60° F.

Mortalité et développement à 75° F. et 80° F., dans des conditions hygrométriques variables

L'analyse de variance du Tableau IV indique que chez *D. polytomum* la mortalité durant la nymphose fut grandement affectée par les conditions de température et d'humidité que nous avons en incubateur. La moyenne de mortalité pour chacun des trois traitements fut comme suit: à 85° F., 85% R.H., mortalité 43.8%; à 75° F., 85% R.H., mortalité 40%; à 75° F., 56% R.H., mortalité 54.7%. Pour être significative, la différence entre ces moyennes doit être égale à:

$$\frac{9.2}{\sqrt{24}} \times 1.414 \times 2.03 = 5.4$$

Comme on le voit, la différence de mortalité aux températures de 75° F. et 85° F., en milieu très humide est négligeable. De fait, l'humidité relative, plus que la chaleur, influence la mortalité: une température de 85° F., en

milieu sec est nettement fatale à *Diprion*. Toutefois, si l'atmosphère est humide, le pourcentage de mortalité sera moindre à 85° F. qu'à 75° F. (température optimum) si cette dernière est associée à une atmosphère trop sèche (56% R.H.).

De plus nous avons vu précédemment que l'influence des températures d'hibernation sur le taux de mortalité est en rapport étroit avec le pourcentage d'humidité durant la nymphose. A des températures d'hibernation supérieures au point de congélation, l'humidité en incubateur n'influence pas la mortalité. Mais, à environ 32° F. ou à des températures inférieures, le pourcentage de mortalité est beaucoup plus élevé en milieu sec.

A la lumière de ces expériences on peut donc conclure que le milieu optimum de nymphose pour *Diprion polytomum* est de 75° F. à 80° F., avec une humidité relative de 85% ou plus.

A 75° F., dans un milieu très sec, Tableau VII, le pourcentage d'émergence chez *Diprion polytomum* n'est pas influencé par les températures d'hibernation. Cependant, lorsque la température de nymphose devient plus élevée (80° F.), il semblerait que la rupture de la diapause soit plus difficile si l'hibernation a eu lieu à de basses températures (15° F.). En effet, alors que j'obtins 50.4% de développement et 17.2% d'émergence après une hibernation à 32° F., il n'y eut, lorsque l'hibernation se fit aux environs de 15° F., seulement 9.9% de développement et 3.2% d'émergence.

En 1939, six lots placés à 75° F. et 22% R.H. présentèrent 27.9% de développement. En 1941, huit lots à la même température mais à 85% R.H. montrèrent un développement de 13% seulement. Ces différences peuvent s'expliquer en grande partie par l'origine différente des individus et par des conditions dissemblables de milieu au cours des deux années, car normalement on devrait s'attendre que le développement soit moindre à 22% d'humidité relative. Faut-il conclure de tout cela que les variations hygrométriques n'ont pas d'influence sur la rupture de la diapause? Il semble que oui, tout au moins pour tous les cas où les écarts dans l'état hygrométrique ne sont pas trop considérables. La preuve de ceci, c'est qu'en 1941 j'avais deux incubateurs réglés à 75° F., dont un à 85% R.H. et l'autre à 56% R.H. Il est vrai que la différence dans les conditions hygrométriques fut loin d'être aussi grande que pour les cas relatés plus haut, mais les résultats, Tableaux V et VI, démontrent tout de même de façon très nette que cette différence de 30% d'humidité relative n'affecte en rien l'émergence, que l'hibernation ait eu lieu à 34° F. ou 45° F., et qu'elle ait duré 84 ou 112 jours.

D'autre part, si l'état hygrométrique de l'atmosphère joue un rôle peu important dans la rupture de la diapause, par contre une forte déficience en humidité influencera considérablement l'émergence des adultes. En effet, en 1939, à une température de 75° F., l'émergence ne fut que de 10.1% à 25% d'humidité relative, alors qu'en 1941 avec 85% R.H., elle atteignait 10.6%. Comme j'avais 27.9% de développement en 1939 et 13% en 1941, ceci veut donc dire que dans une atmosphère très sèche, 17.8% des individus moururent à l'état de pupes et surtout d'adultes dans leur cocon contre seule-

ment 3.2% à 85% R.H. Ceci s'explique par le fait qu'en milieu sec le cocon est beaucoup plus dur et souvent l'adulte ne peut réussir à couper sa capsule de sortie. Dans l'incubateur de 80° F., 22% R.H., le nombre d'adultes morts dans leur cocon fut encore plus considérable. Ainsi, sur un total de 96 cocons ouverts après 12 semaines à 34° F., suivi de 60 jours à 80° F., 22% R.H., il y avait 46 adultes morts; 28 de ces adultes avaient commencé à couper leur trou de sortie mais moururent avant d'avoir terminé leur travail. Le même état de chose s'est répété partout où, en 1939, le développement fut réellement significatif. C'est ce qui explique une si grande marge entre le pourcentage de développement et d'émergence au Tableau VII.

INFLUENCE DE VARIATIONS BRUSQUES DE TEMPÉRATURE

D'après certains auteurs, non seulement le froid, mais toute variation extrême des températures normales aiderait à provoquer la rupture de la diapause. Ces variations brusques ou chocs thermiques, comme les appelle Cousin (30), amèneraient la reprise du développement là où des changements graduels ne produisent que peu ou pas d'effet.

Afin de vérifier si de telles variations sont effectives chez *D. polytomum*, les traitements suivants furent donc essayés en 1940-1941.

C. Dès leur arrivée au laboratoire, après avoir subi dans leur milieu naturel des températures d'environ 45° F. à 50° F., divers lots de cocons furent placés pendant deux semaines dans l'incubateur réglé à 75° F. et 85% R.H. Ensuite, ils furent transférés à 34° F., pour des périodes variant de 6 à 16 semaines puis ramenés directement à 75° F., pour 60 jours.

E. A la fin de l'hibernation, variations brusques de 34° F., à la température d'incubateur, mais sans exposition à 75° F., au début de l'hibernation.

F. Quatre jours avant la fin de l'hibernation, traités comme suit: deux jours à 75° F., suivi de deux jours à 34° F., puis retournés directement à l'incubateur de 75° F., pour la période de nymphose (60 jours).

G. Au début de l'hibernation, deux jours à 34° F., suivi de deux jours à 75° F., puis retournés à 34° F., pour les périodes habituelles variant de 6 à 16 semaines. A la fin de l'hibernation, passage graduel à 75° F., 85% R.H.

Mortalité durant la nymphose

Le pourcentage de mortalité constaté pour les traitements mentionnés ci-haut est présenté au Tableau VIII et l'analyse statistique en est faite au Tableau IX.

Cette analyse prouve que le nombre de jours en hibernation influence le pourcentage de mortalité durant la nymphose, mais ceci n'a rien d'anormal et ne présente qu'un intérêt secondaire. Ce qui nous intéresse dans le moment, c'est de connaître l'influence des variations brusques de température et, comme on le voit par la valeur de *F* qui n'est que 1.15 comparée à 3.29

TABLEAU VIII

Diprion polytomum, INFLUENCE DE VARIATIONS BRUSQUES DE TEMPÉRATURE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Hibernation en jours	Types de variations de température			
	C	E	F	G
	Mortalité			
42	17.9	35.9	17.5	41.3
56	39.0	34.2	37.5	54.4
84	49.5	30.7	53.7	38.9
112	68.5	46.1	53.8	40.7

TABLEAU IX

Diprion polytomum, ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau VIII)

Source de variation	D.L.	Variance
Blocs	1	.21
Traitements	15	351.39**
Jours	3	784.13**
Variations	3	86.76
Jours X variations	9	295.36**
Erreur	15	75.13
Total	31	Erreur standard = 8.668

** Dépasse "erreur" variance, niveau significatif de 1%.

pour $P = 5\%$, aucune des différentes variations expérimentées n'a été par elle-même la cause d'une mortalité réellement plus élevée. Par ailleurs, dans le cas de variations brusques de la température d'hibernation à celle de nymphe, traitements C, E, F, le pourcentage de mortalité augmente avec la durée du séjour à 34° F.

En outre, si l'on compare ces pourcentages de mortalité, Tableau VIII, avec celui obtenu après une hibernation à 34° F. (39.98%) on constate que les variations brusques de température au début ou à la fin de l'hibernation ne réduisent pas la vitalité de *Diprion polytomum* lors de la période de nymphe.

Émergence

D'après les résultats présentés au Tableau X, il n'y a aucun doute qu'un stage prolongé à de hautes températures au début de l'hibernation, après que l'insecte est entré en diapause, rend beaucoup plus difficile la rupture de

la diapause lorsque l'insecte est ensuite placé dans un milieu facilitant normalement la nymphose. Par contre, aucun des traitements *E*, *F*, ou *G* ne provoque un pourcentage d'émergence supérieur aux autres.

TABLEAU X

Diprion polytomum, INFLUENCE DE VARIATIONS BRUSQUES DE TEMPÉRATURE SUR LE POURCENTAGE D'ÉMERGENCE

Hibernation en jours	Types de variations de température			
	<i>C</i>	<i>E</i>	<i>F</i>	<i>G</i>
	Émergence			
84	2.5	25.3	11.8	10.0
112	2.0	15.0	23.5	20.2

La comparaison du développement obtenu après un passage graduel des températures d'hibernation à celles de la nymphose, Tableau V, avec le développement après des variations brusques de température est intéressante. Pour être significative, la différence dans le pourcentage d'émergence entre les deux groupes de traitements comparés doit être au moins de:

$$2.36 \sqrt{\left(\frac{4.104}{\sqrt{8}}\right)^2 + \left(\frac{4.801}{\sqrt{4}}\right)^2} = 2.36 \times 2.8 = 6.63$$

Lorsque le changement de la température d'hibernation à celle de la nymphose fut graduel, nous avons 10.9% d'émergence, Tableau V, alors qu'avec les traitements *E*, *F*, *G*, il fut de 20.2, 17.7, et 15.1 respectivement. Les variations brusques de température à la fin de la diapause, traitements *E* et *F*, provoqueront donc un développement beaucoup plus grand que les changements graduels dans la température, mais les variations brusques au début de l'hibernation n'ont, comme on pouvait s'y attendre, aucune supériorité marquée sur les changements graduels.

NYPHOSE SANS EXPOSITION AUX BASSES TEMPÉRATURES

Lors de leur arrivée au laboratoire 300 cocons furent placés dans l'incubateur à 75° F., 85% R.H., sans passer par les températures d'hibernation. A tous les 15 jours, 50 cocons furent ouverts et l'état des individus noté. Comme les résultats ne présentèrent rien de particulier, inutile de les donner ici en détail. Signalons toutefois, qu'aucun développement ne fut observé.

On peut donc conclure de ces quelques expériences, qu'une fois que la larve de *Diprion polytomum* est entrée en diapause, il faut nécessairement un stage aux basses températures d'hibernation pour déclencher à nouveau le développement.

(La Partie II paraîtra dans le numéro de décembre.)

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 19, SEC. D.

DECEMBER, 1941

NUMBER 12

LA DIAPAUSE CHEZ LES TENTHRÈDES

PARTIE II¹

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Influence d'immersions sur la rupture de la diapause

TECHNIQUE ET CONSIDÉRATIONS GÉNÉRALES

Dans la première partie du présent travail nous avons vu que plusieurs auteurs, entre autres, Townsend (114) et Babcock (4), ont insisté sur l'influence prépondérante de l'immersion préalable des cocons dans l'eau pour amener la rupture de la diapause. Dans leur milieu naturel, les cocons de la mouche à scie européenne de l'épinette, de même que ceux de la mouche à scie du mélèze sont fréquemment en contact avec l'eau. De plus cette eau a un pH généralement acide et l'on peut se demander avec raison si cette condition n'influence pas la mortalité et le développement de la larve dans le cocon. C'est ce que j'ai cherché à vérifier dans une série d'expériences dans lesquelles j'ai utilisé, en plus de l'eau distillée, des solutions acides à des pH de 5 et de 3. Deux acides furent employés pour obtenir ces pH: un inorganique, l'acide sulfurique (H_2SO_4), et l'autre organique, le glycolle ($\text{NH}_2\text{CH}_2\text{COOH}$).

Townsend (114) dans ses recherches sur la pyrale du pommier en était arrivé à la conclusion que des immersions répétées et de courte durée sont plus efficaces pour provoquer la reprise du métabolisme normal après l'hibernation que des immersions longues et moins fréquentes. Les deux sortes d'immersions furent donc mises à l'essai. Pour *D. polytomum* Htg., j'ai fait dans certains cas (Série *a*) trois immersions d'une heure chacune, les lundi, mercredi, et vendredi; alors que dans d'autres cas (Série *b*) je ne fis qu'une immersion de deux heures consécutives le mercredi. Dans le cas de *P. erichsoni* Htg. qui vit généralement dans un milieu plus humide que *D. polytomum*, j'ai procédé aux immersions suivantes: deux heures consécutives le mercredi (Série *b*); quatre heures consécutives le mercredi (Série *c*); deux heures consécutives les lundi, mercredi, et vendredi (Série *d*).

Les individus subissant des immersions dans l'eau distillée furent divisés en trois groupes: 1. Immersions au cours de l'hibernation seulement (34°F.); 2. Immersions durant la nymphose seulement (75°F.); 3. Immersions durant l'hibernation et la nymphose. Dans le cas des solutions acides, à moins d'indication contraire, les immersions ont toujours eu lieu à 34° et 75°F.

Notre façon de procéder dans toutes ces expériences d'immersions forcées consistait à submerger les tubes de verre contenant les cocons dans des

¹ La Partie I a paru dans le numéro de novembre.

bassins d'eau, dont la température était celle du milieu. Après l'opération, ces tubes étaient simplement secoués afin d'enlever toute l'eau possible. Le grand nombre d'expériences que j'avais en marche sur différents genres d'immersions (182 lots de 100 cocons chacun) permettait difficilement un traitement plus minutieux. J'ai vite constaté, cependant, surtout lors des immersions en incubateur, que cette façon de procéder était inadéquate. Le cocon n'étant pas asséché, il restait toujours sur la surface une légère couche de liquide, de sorte qu'en réalité la durée de l'immersion se trouvait être plus longue que la période spécifiée. De plus, les fortes secousses imprimées aux tubes de verre pour enlever l'eau endommageaient les cocons, surtout ceux de *P. erichsoni* dont la paroi est beaucoup moins rigide. Comme conséquence, le fort taux de mortalité constaté dans plusieurs cas pouvait être aussi bien attribué à la manipulation qu'à l'immersion elle-même.

Pour les individus ayant passé 8 et 12 semaines en hibernation, j'ai donc modifié ma technique. Après chaque immersion, les cocons furent asséchés entre deux buvards réduisant ainsi au minimum la mortalité due à la manipulation.

Vitalité de Diprion polytomum dans l'eau

Avant de faire le choix des différentes catégories d'immersion mentionnées plus haut, j'ai cherché au préalable à déterminer la résistance de *D. polytomum* dans l'eau à 34° et 75°F.

Dans nos expériences d'immersions à 34°F., 1500 cocons furent placés dans un bocal qui fut ensuite rempli d'eau. A tous les jours, 50 cocons étaient ouverts et l'état des individus noté. Nous avons procédé de la même façon pour les immersions effectuées à 75°F., mais comme tous nos cocons étaient morts après quatre jours d'immersion, il nous a fallu recommencer l'expérience et procéder à nos examens à des intervalles variant de 5 à 24 heures entre chacun.

Lorsque le cocon a passé plusieurs heures dans l'eau, la larve devient comme asphyxiée et il est souvent impossible au moyen d'un examen sommaire de déterminer si elle est vivante ou morte. Pour être sûr de ne pas faire d'erreur il faut, après que les larves ont été extraites de leur cocon, laisser tous les individus douteux dans l'incubateur au moins une journée avant de les classer définitivement. Après un stage de 24 heures en incubateur, si on presse légèrement la larve asphyxiée mais vivante, on peut, pour le moins, voir remuer les pièces buccales. D'autre part, nous avons noté qu'en incubateur (75°F. et 85% R. H.*), la larve extraite de son cocon peut vivre au moins neuf jours.

Il faut un stage d'au moins 26 jours dans l'eau à 34°F. pour obtenir 100% de mortalité, alors qu'à une température de 75°F. les larves ne résistent que huit jours au maximum (Tableau XI). En conséquence, il est raisonnable de penser qu'en juillet et en août, un stage de trois ou quatre jours dans un milieu saturé d'eau sera fatal à plusieurs larves. Par contre, lors de la fonte

* R.H. = humidité relative.

TABLEAU XI
RÉSISTANCE DE *Diprion polytomum* DANS L'EAU

Immersiones à 34°F.				Immersiones à 75°F.	
Nombre de jours	Mortalité, %	Nombre de jours	Mortalité, %	Nombre d'heures	Mortalité, %
1	5.6	13	62.1	5	2.0
2	8.6	14	63.4	10	4.0
3	11.8	15	66.0	15	7.0
4	15.0	16	69.8	20	7.5
5	25.5	17	67.0	25	12.8
6	31.0	18	72.0	30	20.0
7	37.8	19	74.0	50	24.0
8	41.7	20	78.5	55	30.0
9	45.0	21	82.0	72	85.5
10	52.7	22	86.0	96	96.0
11	57.3	23	92.0	108	100
12	60.6	26	100		

des neiges certains individus pourraient vivre près d'un mois, même s'ils sont complètement submergés.

Constitution de l'humus et acidité des sols forestiers

J'aurais voulu préparer des solutions d'humus au moyen de composés chimiques définis mais, suivant Waksman (119), la chose est impossible, l'humus n'étant pas un composé pouvant être représenté par une formule bien que plusieurs aient été mises en évidence par différents auteurs. D'ailleurs le mot humus n'est pas toujours employé pour désigner les mêmes substances organiques ou préparations. Certains appliquent ce terme à toute la matière organique du sol, alors que d'autres désignent par ce mot seulement cette partie de la matière organique qui est facilement oxydée par certains réactifs.

Quant aux composés chimiques constituant cette matière foncée du sol, un nombre considérable de formules ont été préparées et il semble y avoir eu beaucoup de confusion sur la classification et composition de ce qu'on a appelé, l'humus acide, l'acide humique, etc. Même maintenant, les opinions sont encore partagées sur la nature chimique de l'humus et les groupes qui le constituent. Plusieurs considèrent encore les acides humiques comme les plus importants constituants de l'humus et certains chimistes sont encore convaincus que les acides humiques obtenus en traitant des carbohydrates avec des acides minéraux sont identiques à ceux présents dans les résidus de plantes en décomposition que l'on trouve dans le sol. Après avoir passé en revue la série de formules émises pour représenter l'humus ou l'acide humique, Waksman remarque que le plus important élément de l'humus est l'azote mais que ce corps est généralement omis des différentes formules. Il en conclut que la nature chimique de l'humus n'a pas encore été établie et qu'on ne peut représenter ce complexe par une formule de constituants qui ne sont pas des composés chimiques spécifiques.

Suivant le même auteur, l'humus représente un complexe hétérogène constitué de plusieurs composés d'origine végétale, animale, microbique et de leurs produits de décomposition et de transformation. Bien qu'une foule de composés chimiques peuvent être isolés de l'humus, ce dernier est caractérisé par certains groupes généraux de constituants, variant qualitativement et quantitativement suivant la nature et l'origine de l'humus. On est, par conséquent, justifié de parler de l'humus, non pas comme un complexe chimique, mais comme un état de la matière. De même que les plantes et les substances animales peuvent être sujettes à des analyses chimiques et séparées en plusieurs groupes généraux de composés chimiques possédant des propriétés semblables, de même l'humus peut aussi être divisé en groupes de complexes spécifiques. L'humus originant de la décomposition de matières végétales et animales, la nature des substances qui le constituent change donc continuellement à mesure que progresse la décomposition.

Vu l'impossibilité de préparer, au moyen de composés chimiques définis, des solutions acides qui auraient pu représenter différents types d'humus, il fut décidé d'obtenir les pH désirés au moyen de deux acides: l'acide sulfurique et le glycolle. Dans plusieurs sols, les composés nitrogénés constituent à peu près un tiers de l'humus. Ces composés donnent par hydrolyse des acides aminés et, suivant plusieurs auteurs, la formation de l'humus ne serait que le résultat de la condensation de carbohydrates avec des acides aminés, dont le glycolle.

A quelques exceptions près, nos types d'humus forestiers dans Québec sont toujours acides, le pH pouvant être aussi bas que 3.0. Suivant le Prof. L. Z. Rousseau, de l'École forestière de l'Université Laval, l'humus brut à structure laminée, appelé aussi mor ou duff, a un pH généralement inférieur à 4.5 avec une moyenne de 3.5 à 4.0. L'humus doux ou mull, à structure granuleuse, difficile à distinguer du sol minéral et dont la couche est généralement minime comparée à l'humus brut, a un pH variant de 4.5 à 7.0 avec une moyenne de 5.6 à 6.0.

Préparation de solutions à pH 5 et pH 3

Les solutions que nous avons utilisées furent préparées par le Dr L. Gravel, chimiste, et furent vérifiées au moyen de l'électrode de verre.

Les solutions d'acide sulfurique furent obtenues en diluant dans un gallon d'eau distillée 45 cc. d'acide sulfurique à 0.101 *N* pour les solutions à pH 3 et 45 cc. à 0.00101 *N* pour les solutions d'un pH 5.

Il est impossible d'avoir des solutions de glycolle à pH 3 (117). Pour ce composé les solutions furent donc tamponnées au moyen de l'acide acétique.

Les solutions d'acide sulfurique n'étant pas tamponnées leur pH varie plus facilement sous l'influence d'agents chimiques augmentant ou diminuant le nombre d'ions H^+ . Ces solutions furent donc vérifiées trois fois par semaine et renouvelées chaque fois que le pH était supérieur à 3.

MORTALITÉ DURANT L'HIBERNATION

Diprion polytomum

Les chiffres présentés au Tableau XII et leur analyse au Tableau XIII nous permettent de tirer les conclusions suivantes sur l'effet d'immersions dans différentes sortes de solutions aqueuses au cours de l'hibernation:

TABLEAU XII

Diprion polytomum, INFLUENCE D'IMMERSIONS SUR LE POURCENTAGE DE MORTALITÉ DURANT L'HIBERNATION

Hibernation en jours	Blocs	H ₂ O		H ₂ SO ₄ à pH 3		NH ₂ CH ₂ COOH à pH 3	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
42	I	16	20	20	20	24	16
	II	16	16	24	24	20	20
56	I	0	16	12	36	20	40
	II	20	24	20	40	36	48
84	I	20	28	20	16	28	36
	II	20	32	16	24	32	40
112	I	28	28	12	16	24	24
	II	20	32	16	20	20	28
Moyenne		17.5	24.5	17.5	24.5	25.5	31.5

TABLEAU XIII

ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT L'HIBERNATION

(Voir Tableau XII)

Sources de variation	D.L.	Variance
Blocs	1	161.33
Traitements	23	134.43**
Jours	3	114.33**
Solutions	2	300.00**
Immersion	1	533.33**
Jours × Sol.	6	176.66**
Jours × Imm.	3	141.56**
Sol. × Imm.	2	1.38
Jours × Sol. × Imm.	6	20.90
Erreur	23	19.42
Total	47	Erreur standard = 4.41

Différences significatives: Jours = 3.73
 Solutions = 3.23
 Immersion = 2.64
 Jours × Sol. = 6.46
 Jours × Imm. = 5.27

** Dépasse "erreur" variance, niveau significatif de 1%.

1. La différence significative que l'on constate dans le pourcentage de mortalité après différentes périodes d'hibernation s'explique par les chiffres exceptionnellement élevés pour six et huit semaines d'hibernation chez les individus ayant subi des immersions dans le glyocolle. Normalement, cependant, la durée de l'hibernation n'affecte pas le pourcentage de mortalité, même si, au cours de celle-ci, les cocons subissent des immersions de deux ou trois heures par semaine dans différentes solutions aqueuses.

2. Après hibernation à 34°F., sans immersion (Tableau I*), nous avons obtenu 21.5% de mortalité. Lorsque l'hibernation comporte des immersions de deux heures par semaine dans l'eau ou dans une solution d'acide sulfurique à pH 3, nous avons exactement 21.0% (Tableau XII). C'est dire que celles-ci ne sont aucunement défavorables à la larve. D'autre part, les immersions dans le glyocolle, avec 25.1% de mortalité, sont décidément incompatibles aux individus en hibernation.

3. Un séjour de deux heures par semaine dans des solutions aqueuses (20.2% de mortalité) n'affecte pas la larve, mais si la durée des immersions est portée à trois heures, nous créons alors un milieu défavorable puisque nous avons 26.8% de mortalité et que la différence significative n'est que de 2.6%.

4. Lorsque le stage à 34°F., est relativement court, 42 jours, la nature de la solution aqueuse employée de même que la durée des immersions n'affectent pas le pourcentage de mortalité. Mais lorsque la période d'hibernation est supérieure à six semaines, les immersions dans les solutions de glyocolle, que ce soit de deux ou trois heures, de même que toutes les immersions de trois heures en général, que ce soit dans l'eau, l'acide sulfurique ou le glyocolle, sont fatales à une proportion beaucoup plus considérable des sujets.

Pristiphora erichsoni

Le résultat de nos observations nous porte à croire qu'à l'encontre de ce qui a été constaté pour *D. polytomum*, la nature de la solution aqueuse n'influence pas la mortalité chez la mouche à scie du mélèze. Mais vu le petit nombre d'individus prélevé sur chaque lot et examiné à la fin de l'hibernation, il ne fut pas possible de faire une analyse de variance aussi complète que celle présentée au Tableau XIII pour *D. polytomum*. Toutefois pour les simples immersions dans l'eau, vu que durant l'hibernation quatre lots reçurent les mêmes traitements, j'ai pu, en groupant mes résultats tels que donnés au Tableau XIV, en faire l'analyse statistique et déterminer l'influence des immersions de différentes durées pour différentes périodes d'hibernation.

Bien que les variations dans le pourcentage de mortalité après différentes périodes d'hibernation soient assez considérables (Tableau XIV), l'analyse de variance du Tableau XV nous prouve que ces différences ne sont pas suffisantes pour nous donner raison d'attacher une importance quelconque aux variations enregistrées. Il en est de même pour l'interaction de la période d'hibernation sur la durée des immersions.

*Dans la Partie I.

TABLEAU XIV

Pristiphora erichsoni, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE DE MORTALITÉ DURANT L'HIBERNATION

Traitements	Répétitions	Hibernation en jours			
		42	56	84	112
Aucune immersion	1	12	0	20	10
	2	4	50	10	20
	3	24	10	10	20
	4	16	20	20	20
2 hres d'immersion	1	20	30	60	30
	2	24	50	50	20
	3	24	10	20	20
	4	24	0	30	30
4 hres d'immersion	1	28	20	40	10
	2	28	70	30	40
	3	28	0	30	40
	4	24	20	20	10
6 hres d'immersion	1	0	50	50	40
	2	8	40	60	60
	3	32	60	40	20
	4	24	70	40	10
Moyenne		20	31.2	33.1	24.4

TABLEAU XV

ANALYSE DE VARIANCE SUR LA MORTALITÉ DE *Pristiphora erichsoni*
DURANT L'HIBERNATION

(Voir Tableau XIV)

Sources de variation	D.L.	Variance
Répétitions	3	473.42
Traitements	15	535.58**
Jours	3	593.75
Immersion	3	1122.92**
Jours × Imm.	9	320.42
Erreur	45	212.26
Total	63	Erreur standard = 14.569

** Dépasse "erreur" variance, niveau significatif de 1%.

Par contre les variations associées aux immersions sont très prononcées et nous avons une probabilité de beaucoup inférieure à 1%. Pour être significatives, les différences entre les moyennes de mortalité doivent avoir un minimum de:

$$\left(\frac{14.72 \sqrt{2}}{\sqrt{16}} \right) 1.96 = 10.$$

Une simple inspection du Tableau XIV nous permet d'affirmer que *Pristiphora erichsoni* peut aussi bien supporter quatre heures que deux heures d'immersions continues par semaine. Mais il n'en est pas de même pour trois semaines de deux heures chacune par semaine, alors que le pourcentage de mortalité monte à 37%. De plus, il semble que la larve s'accommode beaucoup mieux d'une hibernation sans contact avec l'eau (16.6% de mortalité) qu'aux hibernations avec immersions, même si elles ne sont que de deux heures par semaine.

Ces résultats, toutefois, ne sont pas très concluants, parce que, comme je l'ai déjà fait remarquer, le cocon de la mouche à scie du mélèze est très flexible, et dans le cas des lots ayant subi des immersions, il est probable qu'une bonne partie de la mortalité puisse être attribuée à la manipulation. C'est ce qui expliquerait qu'entre quatre et six heures d'immersion nous ayons une différence d'environ 10% de mortalité; le dernier traitement ayant nécessité trois manipulations contre une seulement lorsqu'il s'est agit de quatre heures consécutives d'immersion. D'ailleurs si l'on considère que *P. erichsoni* vit normalement dans un milieu plus humide que *D. polytomum*, il serait surprenant que *Pristiphora* soit affecté par de courtes immersions dans l'eau alors que *Diprion* ne l'est pas.

MORTALITÉ DURANT LA NYMPHOSE

La grande majorité des larves de *Pristiphora erichsoni* ayant subi des immersions dans des solutions aqueuses étaient mortes après 60 jours en incubateur. Même lorsque les cocons furent asséchés en les secouant légèrement entre deux buvards, comme ce fut le cas pour certains lots ayant passé 12 semaines d'hibernation, le pourcentage de larves vivantes à la fin des expériences fut encore inférieur à 3%. Ces résultats ne peuvent pas être attribués aux conditions de milieu, mais plutôt à la manipulation. La technique employée, immersion des tubes suivie de fortes secousses pour enlever l'eau, s'est avérée tout à fait défectueuse pour la mouche à scie du mélèze. Heureusement, il n'en fut pas de même pour *Diprion* et les résultats présentés aux Tableaux XVI, XVIII, et XX nous ont permis des constatations intéressantes.

Immersion dans l'eau

L'analyse statistique du Tableau XVII démontre que:

1. Lorsque le cocon est en contact avec l'eau, le taux de mortalité en incubateur est nettement plus élevé après 56 jours d'hibernation (52.6%) qu'après 42 jours (37.3%). D'autre part, les différences observées lorsque l'hibernation varie de 56 à 116 jours sont négligeables. Dans les conditions naturelles, la diapause, lorsque diapause il y a, est généralement supérieure à

huit semaines. Au printemps, lorsque la température devient favorable à la réactivation, la résistance de la larve dont le cocon est souvent en contact avec l'eau ne serait donc pas influencée par la durée de l'hibernation.

TABLEAU XVI

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	Imm. à 34°F.		Imm. à 75°F.		Imm. à 34° et 75°F.	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
I	42	51.2	30.1	51.6	46.1	38.8	26.8
	56	34.7	34.0	55.8	52.6	22.8	100
	84	39.7	49.4	59.2	43.2	42.7	96.9
	112	45.2	45.7	50.5	46.3	55.7	39.5
II	42	36.6	30.1	41.9	43.5	25.0	26.2
	56	37.0	67.6	45.5	48.2	32.9	100
	84	40.2	44.2	59.7	42.3	41.4	90.7
	112	57.7	51.5	42.8	43.3	52.6	44.0
Moyenne		42.8	44.1	50.9	45.6	39.0	65.5

Note: Nymphose à 75°F. et 85% R.H.

TABLEAU XVII

ANALYSE DE VARIANCE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XVI)

Sources de variation	D.L.	Variance
Blocs	1	3.85
Traitements	23	591.99**
Jours	3	690.66**
Immersion	2	312.12**
Heures	1	682.52**
Jours × Imm.	6	349.62**
Jours × Hres	3	887.66**
Imm. × Hres	2	1146.06**
Jours × Imm. × Hres	6	538.27**
Erreur	23	48.15
Total	47	Erreur standard = 6.94

Différences significatives: Jours = 5.85
 Immersion = 5.08
 Heures = 4.13
 Jours × Imm. = 10.16
 Jours × Hres = 8.28
 Imm. × Hres = 7.18

** Dépasse "erreur" variance, niveau significatif de 1%.

TABLEAU XVIII

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POUR-CENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	H ₂ SO ₄		NH ₂ CH ₂ COOH	
		pH 5	pH 3	pH 5	pH 3
I	42	17.8	59.4	76.5	47.7
	56	37.2	30.3	35.7	63.5
	84	54.2	32.6	54.6	75.5
	112	47.4	47.1	60.6	74.0
II	42	21.5	34.6	31.5	48.0
	56	33.7	27.1	30.6	47.8
	84	48.4	36.9	55.7	93.8
	112	48.5	35.4	63.0	67.0
Moyenne		38.6	37.9	51.0	64.7

Note: Immersions à 34° et 75°F., deux heures consécutives chaque mercredi.

2. Il n'est pas douteux que le contact de l'eau durant l'hibernation et la nymphose entraîne la mort d'un plus grand nombre d'individus (52%) que si le contact ne se fait que durant l'hibernation (43%). Il semble de plus évident que ce sont les immersions durant la nymphose qui sont particulièrement défavorables, la mortalité étant presque aussi élevée (48%) si les immersions n'ont eu lieu qu'à 75°F., que si elles sont produites à 34° et 75°F. Par contre, l'insecte paraît mieux résister au contact de l'eau au cours de l'hibernation que durant la nymphose et, comme question de fait, les immersions de deux et trois heures durant l'hibernation n'affectent en rien le taux de mortalité durant la nymphose. En effet, lorsque la larve n'a eu aucun contact avec l'eau au cours de l'hibernation et de la nymphose, nous n'avons eu que 40% de mortalité après 60 jours d'incubation à 75° et 85% R.H. Ceci ne représente que 3% de moins que lorsqu'il y a immersion à 34°F. et cette légère différence n'a rien de significatif.

3. Si les immersions n'ont lieu que durant l'hibernation ou la nymphose, la larve supporte également bien les immersions de deux et de trois heures par semaine. Mais, lorsque le cocon subit le contact de l'eau au cours de ces deux phases vitales, la larve offre alors moins de résistance à trois immersions d'une heure chacune par semaine qu'à une seule immersion de deux heures consécutives.

Immersion dans des solutions acides

Toutes ces immersions eurent lieu tant en incubateur qu'à 34°F. sauf quelques exceptions qui sont indiquées plus loin.

Les faits saillants qui ressortent des expériences comportant des immersions dans des solutions acides sont les suivants:

1. Comme pour les immersions dans l'eau, le taux de mortalité est en relation étroite avec la durée du stage à 34°F., et dans le cas présent il est plus élevé après 12 et 16 semaines qu'après six et huit semaines d'hibernation.

2. Si l'on compare les résultats des Tableaux XVI et XVIII, on constate d'une part que *D. Polytomum* réagit aussi bien aux immersions dans les solutions d'acide sulfurique (38% de mortalité) qu'aux immersions d'eau pure (39%). D'autre part, les immersions dans les solutions de glyocolle provoquent un plus fort pourcentage de mortalité (58%), et la différence avec l'acide sulfurique est très marquée (Tableau XIX). D'après le Dr J. Ls. Tremblay, professeur de biologie à Laval, le glyocolle serait en l'occurrence toxique.

TABLEAU XIX

ANALYSE DE VARIANCE SUR LA MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XVIII)

Sources de variation	D.L.	Variance
Blocs	1	256.50
Traitements	15	529.70**
Jours	3	682.18**
Acides	1	3069.36**
pH	1	336.70
Jrs × Ac.	3	75.77
Jrs × pH	3	32.64
Ac. × pH	1	408.98
Jrs × Ac. × pH	3	586.23**
Erreur	15	100.56
Total	31	Erreur standard = 10.03

Différences significatives: Jours = 10.68
Acides = 7.56

** Dépasse "erreur" variance, niveau significatif de 1%.

3. L'analyse de variance des Tableaux XIX et XXI démontre que par lui-même le pH n'a aucun effet mortel sur l'individu dans le cocon. La différence significative de 14.7% de mortalité que l'on note entre les immersions dans les solutions de glyocolle à pH 5 et pH 3 ne peut pas être attribuée au pH, mais bien à la concentration d'une substance qui est contraire à la larve.

4. Aucune des interactions jours sur acides et acides sur pH ne diminue de façon sensible la vitalité des individus.

5. Après deux heures consécutives d'immersion hebdomadaire dans une solution d'acide sulfurique à pH 3, le taux de mortalité est de 38% (Tableau XX). Si les immersions sont portées à trois, d'une heure chacune par semaine, la proportion des larves mortes atteint 76%. Dans les solutions de

glycocolle, la différence de mortalité est aussi très prononcée, soit de 64.7 et 93.5%. D'après notre analyse du Tableau XXI, une différence supérieure à :

$$\frac{16.07}{\sqrt{8}} \times 1.414 \times 2.01 = 16.2$$

est significative. Comme pour les immersions dans l'eau, on peut donc conclure que l'augmentation dans le taux de mortalité est en rapport direct avec la durée de l'immersion.

TABLEAU XX

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

Blocs	Jours à 34°F.	H ₂ SO ₄					NH ₂ CH ₂ COOH		
		pH 5	pH 3				pH 5	pH 3	
		2 hres	2 hres	3 hres	2 hres†	2 hres‡	2 hres	2 hres	3 hres
I	42	17.8	59.4	89.7	16.4	17.0	76.5	47.7	100
	56	37.2	30.3	88.6	27.8	71.5	35.7	63.5	100
	84	54.2	32.6	71.7	35.1	57.2	54.6	75.5	100
	112	47.4	47.1	61.5	43.2	49.4	60.6	74.0	72.4
II	42	21.5	34.6	85.5	16.0	13.6	31.5	48.0	100
	56	36.7	27.1	89.4	34.4	72.5	30.6	47.8	100
	84	48.4	36.9	63.5	48.4	54.5	55.7	93.8	100
	112	48.5	35.4	59.1	40.4	50.0	63.0	67.0	75.5
Moyenne		38.59	37.93	76.13	32.71	48.21	51.03	64.66	93.49

Note: Nymphe à 75°F. et 85% R.H.

† Immersions à 34°F. seulement.

‡ Immersions à 75°F. seulement.

TABLEAU XXI

ANALYSE DE VARIANCE SUR LE POURCENTAGE DE MORTALITÉ DURANT LA NYMPHOSE

(Voir Tableau XX)

Sources de variation	D.L.	Variance
Blocs	1	124.60
Jours	3	449.99
Traitements	7	3588.01**
Erreur	52	258.39
Total	63	Erreur standard = 16.07

** Dépasse "erreur" variance, niveau significatif de 1%.

6. Lorsque les cocons sont immergés dans les solutions d'acide sulfurique durant l'hibernation seulement, le taux de mortalité est de 32.7%, contre 48.2% si l'immersion ne se produit que durant la nymphose. Ceci, encore, confirme ce que nous avons déjà constaté dans le cas des immersions dans l'eau distillée, à savoir que les larves succombent en plus grand nombre si les cocons sont en contact avec l'eau durant la nymphose que durant la diapause.

ÉMERGENCE

Dans plusieurs cas, les immersions durant l'hibernation dans l'eau et les solutions d'acide sulfurique, de même que les immersions durant l'hibernation et la nymphose dans l'acide sulfurique réduisent la diapause d'un mois. En effet, alors qu'il n'y eut aucune émergence chez les lots placés dans l'incubateur de 75°F. et 85% R.H. après huit semaines d'hibernation sans contact avec l'eau, pour les trois traitements mentionnés plus haut, nous avons respectivement 5, 6, et 8% d'émergence. Dans le cas d'immersions de deux heures à 34° et 75°F. dans des solutions d'acide sulfurique, nous avons donc eu presque autant d'émergence après huit semaines d'hibernation qu'après 12 semaines lorsque les cocons ne sont pas venus en contact avec des solutions aqueuses.

A trois exceptions près, nous n'avons noté aucune reprise de développement après huit semaines à 34°F. pour tous les autres types d'immersion. C'est pourquoi dans les remarques qui suivent, je considère l'effet de différents traitements sur la rupture de la diapause seulement après 12 et 16 semaines d'hibernation.

Vu que dans presque tous les cas où il y a eu reprise de développement, il en est résulté l'éclosion de l'adulte du cocon, le taux d'émergence a été utilisé comme indice d'efficacité de tel ou tel type d'immersion sur la réactivation des larves. Dans de rares cas, bien qu'il y a eu rupture de la diapause et de la nymphose, les conditions de milieu étant défavorables aux pupes et adultes nouvellement formés, il en est résulté une mortalité anormale durant ces deux stages. Ceci s'est présenté notamment après 8 et 12 semaines chez les lots subissant des immersions à 34° et 75°F., dans des solutions de glycolle à pH 5. Dans le cas des lots ayant passé 12 semaines en hibernation, les sujets morts à l'état de pupes ou d'adultes dans le cocon représentaient 23% des individus vivants et éclos à la fin des expériences.

Pour éviter toute longueur inutile, résumons ensemble les résultats obtenus après des immersions dans l'eau et dans des solutions acides, tels que présentés et analysés aux Tableaux XXII à XXVII.

1. Dans tous les cas, le nombre de larves qui se nymphosent est plus grand après 16 que 12 semaines d'hibernation.

2. Le taux d'émergence est également plus considérable avec des immersions uniques de deux heures consécutives chaque semaine qu'avec trois immersions hebdomadaires d'une heure chacune.

TABLEAU XXII

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS L'EAU SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	Imm. à 34°F.		Imm. à 75°F.		Imm. à 34°F. et 75°F.	
		2 hres	3 hres	2 hres	3 hres	2 hres	3 hres
I	84	21.4	4.1	6.5	3.6	0	0
	112	10.9	9.8	17.4	5.9	14.3	5.8
II	84	17.2	13.2	0	3.8	0	0
	112	26.8	10.6	7.1	9.8	11.4	4.3
Moyenne		19.1	9.4	7.8	5.8	6.4	2.5

Note: Nymphose à 75°F. et 85% R.H.

TABLEAU XXIII

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau XXII)

Sources de variation	D.L.	Variance
Blocs	1	0.84
Traitements	11	84.91*
Jours	1	172.27*
Immersion	2	209.13*
Heures	1	160.85*
Jrs × Imm.	2	37.50
Jrs × Hres	1	18.74
Imm. × Hres	2	31.80
Jrs × Imm. × Hres	2	12.72
Erreur	11	23.93
Total	23	Erreur standard = 4.89

Différences significatives: Jours et heures = 4.41
Immersion = 5.41

* Dépasse "erreur" variance, niveau significatif de 5%.

TABLEAU XXIV

Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	H ₂ SO ₄		NH ₂ CH ₂ COOH	
		pH 5	pH 3	pH 5	pH 3
I	84	9.3	30.6	6.8	0
	112	29.5	34.8	25.6	0
II	84	2.0	24.1	7.1	0
	112	26.3	26.7	16.2	6.1
Moyenne		16.5	29.0	13.9	1.5

TABLEAU XXV
ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE
(Voir Tableau XXIV)

Sources de variation	D.L.	Variance
Blocs	1	49.35
Traitements	7	320.01**
Jours	1	454.75**
Acides	1	922.64**
pH	1	0.02
Jrs × Ac.	1	18.71
Ac. × pH	1	608.86**
Jrs × pH	1	221.28**
Jrs × Ac. × pH	1	13.79
Erreur	7	14.45
Total	15	Erreur standard = 3.801

** Dépasse "erreur" variance, niveau significatif de 1%.

TABLEAU XXVI
Diprion polytomum, INFLUENCE D'IMMERSIONS DANS DES SOLUTIONS ACIDES SUR LE POURCENTAGE D'ÉMERGENCE

Blocs	Jours à 34°F.	H ₂ SO ₄					NH ₂ CH ₂ COOH		
		pH 5	pH 3				pH 5	pH 3	
		2 hres	2 hres	3 hres	2 hres†	2 hres‡	2 hres	2 hres	3 hres
I	84	9.3	30.6	0	21.3	0	6.8	0	0
	112	29.5	34.8	21.6	23.6	17.9	25.6	0	3.7
II	84	2.0	24.1	0	13.7	2.5	7.1	0	0
	112	26.3	26.7	20.5	32.2	8.9	16.2	6.1	0
Moyenne		16.8	29.0	10.5	22.7	7.3	13.9	1.5	0.9

† Immersion à 34°F. seulement.

‡ Immersion à 75°F. seulement.

TABLEAU XXVII
ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Voir Tableau XXVI)

Sources de variation	D.L.	Variance
Blocs	1	46.08
Jours	1	970.21**
Traitements	7	389.95**
Erreur	22	30.09
Total	31	Erreur standard = 5.49

** Dépasse "erreur" variance, niveau significatif de 1%.

3. Lorsque les immersions se font dans l'eau pure, il n'y a pas de différence marquée dans la reprise du développement, que ces immersions se produisent durant la nymphose seulement ou bien durant l'hibernation et la nymphose; 6.8 et 4.5% d'émergence. Par contre, si les immersions ont lieu à 34°F. seulement, la diapause est plus facilement rompue et l'émergence est alors de 14.3%.

4. Lorsque les immersions se font dans une solution d'acide sulfurique, les résultats sont tout autres. D'abord si elles ont lieu à 75°F. seulement, le taux d'émergence (7.3%) est nettement inférieur à celui des immersions à 34°F., de même qu'à celles de 34° et 75°F. (Tableau XXVI). Ensuite, la réactivation maximum n'est pas obtenue ici après les immersions à 34°F., avec 22.7% d'émergence, mais bien après le traitement comportant des immersions à 34° et 75°F., avec 29% d'émergence. Cependant, cette différence entre les deux traitements n'est pas assez considérable pour démontrer la supériorité d'un traitement sur l'autre.

5. Après deux heures par semaine de séjour dans l'eau à 34°F., suivi d'un stage, de 60 jours en incubateur, 19.1% des larves se nymphosent, contre 10.6% seulement s'il n'y a pas eu immersion à 34°F. (Tableau V*). Les erreurs standards des deux groupes d'expériences (Tableaux XXIII et VI*) sont 4.89 et 4.10 avec un minimum de sept degrés libres. Pour être significative la différence entre nos deux moyennes devrait être d'au moins:

$$2.36\sqrt{\left(\frac{4.89}{\sqrt{4}}\right)^2 + \left(\frac{4.10}{\sqrt{4}}\right)^2} = 7.5$$

Il n'y a donc pas de doute qu'après des immersions de deux heures par semaine durant l'hibernation, la diapause est plus facilement rompue que si le cocon ne vient pas en contact avec l'eau.

Si l'on compare maintenant les résultats obtenus après deux heures d'immersions dans l'eau pure à 34°F., avec ceux de deux heures d'immersions à 34° et 75°F., dans des solutions d'acide sulfurique à pH 3 on observe une différence de (29 - 19.1) 9.9% dans le nombre d'individus qui reprennent leur développement lorsque placés par la suite en incubateur. Nous avons une différence significative lorsqu'elle est supérieure à:

$$2.20\sqrt{\left(\frac{5.89}{\sqrt{4}}\right)^2 + \left(\frac{4.89}{\sqrt{4}}\right)^2} = 8.4$$

Les immersions dans des solutions d'acide sulfurique à pH 3 durant l'hibernation et la nymphose provoquent donc une réactivation plus grande que les immersions dans l'eau pure.

6. La variance pour les acides, Tableau XXV, est supérieure au niveau significatif de 1%, démontrant ainsi la supériorité de l'acide sulfurique sur le glycolle. Cependant, lorsque le pH n'est que de 5, la concentration n'est pas suffisante pour mettre en évidence l'action contraire des deux acides. C'est pourquoi au Tableau XXVI, la différence de développement pour les

* Dans la Partie I.

deux solutions à pH 5 n'est pas significative. Mais à un pH de 3, après deux heures d'immersion par semaine à 34° et 75°F., le contraste est frappant, soit 29% d'émergence pour l'acide sulfurique et 1.5% seulement pour le glyco-colle.

7. Les chiffres du Tableau XXVI montrent que le pH agit fortement sur la rupture de la diapause; le taux d'émergence étant de 16.8% après immersion dans une solution d'acide sulfurique à pH 5, et 29% après immersion à pH 3. Il est vrai que d'après notre analyse du Tableau XXV, l'effet du pH serait nul, mais dans ce cas notre analyse, du moins en ce qui concerne le pH, est faussée par les résultats du glyco-colle à pH 3 où le développement fut à peu près nul. C'est pour des cas de ce genre (Tableau XXIV), que Cochran (29) écrivait que lorsque les différences entre différents traitements sont de plus de 100% ou lorsqu'il y a insuccès partiel de certains traitements, il valait mieux dans l'analyse de variance d'omettre ces traitements. Comme règle générale dans les analyses de routine, Cochran suggère d'omettre les traitements dont les résultats sont continuellement plus du double ou de moins de la moitié du groupe principal de traitements. Il aurait donc été préférable d'éliminer du Tableau XXIV la colonne du glyco-colle à pH 3. J'ai conservé cette colonne pour deux raisons: d'une part, afin de ne pas enlever la symétrie du tableau, ce qui aurait rendu impossible l'analyse de variance pour d'autres sources de variation qui ne sont pas faussées par le peu de développement dans le glyco-colle à pH 3; d'autre part, pour illustrer comment on peut arriver à de fausses interprétations en faisant des analyses de variance de façon mécanique. Au Tableau XXVIII, les résultats avec les solutions de glyco-colle ont été éliminés et le pH nous donne alors une variance très significative, mettant ainsi en évidence l'influence du pH sur la réactivation des larves.

TABLEAU XXVIII

ANALYSE DE VARIANCE SUR LE POURCENTAGE D'ÉMERGENCE

(Basée sur Colonnes 3 et 4 du Tableau XXIV)

Sources de variation	D.L.	Variance
Blocs	1	78.82
Traitements	3	252.68*
Jours	1	329.42*
pH	1	301.42*
Jours × pH	1	127.21
Erreur	3	18.95
Total	7	Erreur standard = 4.353

* Dépasse "erreur" variance, niveau significatif de 5%.

8. D'après l'interaction jours sur pH, Tableau XXV, le développement après 84 jours d'hibernation serait plus considérable à pH 3 qu'à pH 5, tandis qu'après 112 jours un pH de 3 diminuerait l'émergence. Ici, encore, l'analyse est faussée par les résultats du glycolle à pH 3 qui n'auraient pas dû être considérés. En effet, l'analyse du Tableau XXVIII nous prouve que l'interaction jours sur pH n'influence en aucune façon le pourcentage d'émergence.

Action d'immersions dans l'acide sulfurique concentré

Les expériences décrites dans ce chapitre avaient pour but de déterminer l'influence que peut avoir sur la rupture de la diapause: (a) une dessiccation rapide au début de l'hibernation, et (b) l'action de chocs chimiques.

A cet effet, avant ou après les périodes à 34°F., périodes variant comme d'habitude de 42 à 116 jours, les traitements suivants furent expérimentés:

e. Immersion de trois minutes dans une solution à 95% d'acide sulfurique au début de l'hibernation suivie, durant la nymphose, du traitement b comportant trois immersions dans l'eau d'une heure chacune par semaine pour *Diprion*, et du traitement d, soit six heures d'immersion pour *Pristiphora*.

f. Immersion de trois minutes dans l'acide sulfurique concentré à la fin de l'hibernation sans immersion dans l'eau à 75°F. par la suite.

g. Immersion de trois minutes dans l'acide sulfurique à la fin de l'hibernation, suivie des traitements b et d durant la nymphose.

h. Même traitement que e mais avec une durée d'hibernation de 112 jours.

i. Immersion d'une minute dans l'acide sulfurique à la fin de l'hibernation. Aucun contact avec l'eau dans l'incubateur.

j. Immersion de deux minutes dans l'acide sulfurique à la fin de l'hibernation. Aucun contact avec l'eau à 75°F.

Je n'avais d'abord prévu dans mon programme que les immersions e, f, et g, mais vu le fort pourcentage de mortalité qui en résulta, les traitements f et g furent changés pour i et j pour les lots subissant 16 semaines à 34°F.

RÉSISTANCE À L'ACIDE SULFURIQUE CONCENTRÉ

Avant d'adopter les immersions de trois minutes, j'ai auparavant déterminé la résistance de *Diprion* dans l'acide sulfurique concentré. Dix-huit lots de 25 cocons chacun furent d'abord placés dans nos tubes de verre de 25 × 90 mm. Ces tubes furent ensuite plongés individuellement, pour des périodes variant de 1 à 12 minutes, dans un bocal rempli d'acide sulfurique à 95% de façon à ce que les cocons soient complètement submergés par l'acide. Une fois l'immersion terminée, les cocons furent lavés à l'eau, puis immergés pour une heure dans un autre bassin rempli d'eau et finalement transférés pour 24 heures, soit dans l'incubateur à 75°F. et 85% R.H., soit dans le compartiment à 34°F. de notre réfrigérateur. Après ce délai, ils furent ouverts et l'état des larves noté. Les résultats obtenus dans ces expériences sont présentés au Tableau XXIX.

TABLEAU XXIX

RÉSISTANCE DE *Diprion polytomum* DANS L'ACIDE SULFURIQUE CONCENTRÉ

Minutes dans H ₂ SO ₄ conc.	% de mortalité après un stage de 24 heures à:		Minutes dans H ₂ SO ₄ conc.	% de mortalité après un stage de 24 heures à:	
	34°F.	75°F. et 85% R.H.		34°F.	75°F. et 85% R.H.
1	16	40†	6	20	44
2	20	28	8	40	64
3	16	16	10	52	84
4	16	28	12	32	84
5	20	20			

† Dans ce lot, 24% des larves étaient mortes avant l'expérience.

Après 24 heures à 34°F. le nombre de larves mortes n'est pas anormal, même si l'immersion a duré jusqu'à 12 minutes. Par contre, après une immersion de 10 minutes dans l'acide on enregistre une mortalité de plus de 80% dans les cocons, 24 heures après qu'ils ont été placés à 75°F. Il n'y a pas de doute qu'en dépit du lavage dans l'eau, il reste toujours une certaine quantité d'acide d'imprégné dans le cocon. Il faut croire qu'à une basse température, l'action de cet acide est presque nulle, mais à une température élevée et dans un milieu très humide, il continue à agir sur la larve et entraîne une plus forte mortalité.

Au début de mes expériences, j'avais décidé d'adopter pour la durée de mes immersions, la moitié de la période requise pour qu'il y ait mortalité notable par l'acide. Des essais préliminaires m'avaient montré que dans le cas des cocons placés en incubateur après leur immersion dans l'acide sulfurique, le taux de mortalité atteignait 44% après six minutes d'immersion, et c'est à ce moment qu'on commençait à constater l'effet mortel de l'acide; c'est pourquoi j'ai choisi des immersions de trois minutes.

Pour *Pristiphora erichsoni*, je ne fis que deux expériences; une immersion de deux minutes et l'autre de trois minutes dans l'acide sulfurique concentré, suivie du lavage dans l'eau et d'un stage de 24 heures dans l'incubateur. Le lendemain sur un total de 40 cocons, il y avait dans un lot 28% des larves de mortes et dans l'autre 32%.

MORTALITÉ DURANT L'HIBERNATION ET LA NYMPHOSE

Les immersions dans l'acide sulfurique concentré au début de l'hibernation n'affectent pas la vitalité de la larve de *P. erichsoni* (Tableau XXX). Ainsi, la moyenne de mortalité à la fin de l'hibernation pour huit lots ayant passé trois minutes dans l'acide sulfurique concentré au début de leur stage à 34°F. fut de 28.2%. Pour 16 lots n'ayant eu aucun contact avec l'acide sulfurique, la moyenne fut de 16.6%, soit une différence de 11.6%. Cette différence, cependant, n'est pas suffisante pour indiquer une action défavorable de l'acide,

TABLEAU XXX

Pristiphora erichsoni, MORTALITÉ DURANT L'HIBERNATION†

Traitements	Moyenne	E.S.	E.S. _M	E.S. _D	Valeurs de T	
					Obtenue	P = 5%
Trois minutes dans H ₂ SO ₄	28.25	14.2	5.02			
Témoin	16.63	17.17	4.29			
Différence	11.62			6.62	1.76	2.36

† Après trois minutes d'immersion dans l'acide sulfurique concentré.

car l'erreur standard de la différence n'est que de 6.6 nous donnant une valeur de "t" pour laquelle la probabilité est supérieure à 5%.

Les résultats obtenus pour *Pristiphora* s'appliquent aussi à *Diprion*, les immersions de trois minutes dans l'acide sulfurique n'augmentant pas la mortalité au cours de l'hibernation.

Apparemment, à des températures près du point de congélation, l'acide ne s'infiltré que difficilement dans le cocon et la larve est très peu affectée.

Tous les individus, indistinctement d'espèce, ayant subi trois minutes d'immersion dans l'acide sulfurique au début ou à la fin de l'hibernation, et des immersions dans l'eau durant la nymphose, sont morts après 60 jours en incubateur et même, dans certains cas, après 30 jours seulement d'incubation. Il n'y a aucun doute que pour un bon nombre d'individus les immersions dans l'acide furent mortelles. Ainsi, là où il y avait des faiblesses dans le cocon, dues soit à la manipulation (pressions trop fortes) ou à la texture du cocon lui-même, l'acide sulfurique s'infiltré à l'intérieur et provoque des fissures. Alors les larves étaient totalement décomposées ou encore complètement sorties du cocon. Ailleurs, il y avait évidence que l'acide sulfurique concentré était venu directement en contact avec les larves par les plaques noires qu'elles avaient sur le corps. Cependant des larves de *Diprion*, ayant ainsi tout le dos de l'abdomen (excepté les trois derniers segments) complètement noirci par l'acide sulfurique, étaient encore très vivantes lorsque examinées. En dépit du nombre de larves tuées par l'action directe de l'acide sulfurique, il n'en reste pas moins que les causes de la mort de la grande majorité des individus furent la manipulation et les immersions de trois heures dans l'eau pour *Diprion*, et celles de six heures pour *Pristiphora*. En effet, surtout en ce qui concerne *Pristiphora*, nous avons vu au chapitre précédent qu'après ces immersions nous avons remarqué 100% de mortalité.

Lorsque les immersions de trois minutes dans l'acide sulfurique concentré ne sont pas suivies d'immersions dans l'eau en incubateur, nous avons encore pour *Pristiphora* presque 100% de mortalité après 60 jours à 75°F. et 85%

R.H. Par contre, chez *Diprion* il y eut toujours de 10 à 35% des larves de vivantes à la fin des expériences. L'acide pénètre plus facilement le cocon à texture lâche de *Pristiphora* et le lavage à l'eau après l'immersion est moins efficace. Par la suite lorsque ces cocons sont placés en incubateur, les gaz d'acide sulfurique qui se dégagent à l'intérieur du cocon, tuent au moins 65% des larves après 30 jours, et la quasi-totalité après 60 jours.

Vu le fort pourcentage de mortalité survenu après les immersions de trois minutes dans l'acide sulfurique, je décidai, pour les individus de *Diprion* ayant passé 16 semaines à 34°F., d'éliminer les traitements *f* et *g* pour appliquer les traitements *i* et *j*, soit une et deux minutes d'immersions dans l'acide sulfurique. Les résultats sont présentés au Tableau XXXI, ainsi que ceux obtenus après huit semaines d'hibernation et trois minutes d'immersions dans l'acide sulfurique.

TABLEAU XXXI

Diprion polytomum, POURCENTAGE DE MORTALITÉ ET D'ÉMERGENCE APRÈS IMMERSION DANS L'ACIDE SULFURIQUE CONCENTRÉ

Traitements†	Bloc I		Bloc II		Moyenne	
	Mort.	Émer.	Mort.	Émer.	Mort.	Émer.
<i>e</i>	100		100		100	
<i>f</i>	82	12.5	69	14.3	75.5	12.2
<i>g</i>	98		100		99.0	
<i>h</i>	79		81.3	6.2	80.6	3.0
<i>i</i>	61	62.5	65	48.6	62.5	56.0
<i>j</i>	77	36.4	75	48	76.0	41.7

† Hibernation de 12 semaines pour *e*, *f*, et *g*; 16 semaines pour *h*, *i*, et *j*.

Après les trois minutes d'immersion dans l'acide sulfurique au début ou à la fin de l'hibernation, lorsqu'il y eut immersion dans l'eau durant la nymphose, nous avons eu 100% de mortalité pour *e* et *g* et pour le traitement *h* (meilleure manipulation) 80.6%. Lorsqu'il n'y eut pas contact avec l'eau durant la nymphose, traitements *f*, *i*, et *j*, la mortalité fut de: 75.5% après trois minutes d'immersion, 76% après deux minutes, et 62.5% après une minute. Dans ces trois derniers cas, ce n'est pas tant l'acide sulfurique que la manipulation qui fut cause de ce haut pourcentage de mortalité. Car une fois sec, le cocon qui a été plongé dans l'acide sulfurique concentré devient très fragile. A la moindre pression ces cocons s'effritent presque aussi facilement que du papier brûlé. Comme question de fait, à la fin des expériences, il y en avait plusieurs réduits en miettes. Et s'ils n'avaient pas eu à être examinés tous les jours pour l'émergence des adultes, la mortalité aurait été beaucoup moindre.

ÉMERGENCE

Les seuls cocons de la mouche à scie du mélèze contenant des individus vivants à la fin des expériences appartenaient aux lots du traitement *f*, mais aucun adulte n'est éclos de ces cocons.

Des lots de *Diprion* qui, après leur immersion de trois minutes dans l'acide sulfurique, vinrent en contact avec l'eau durant la nymphose, il n'est sorti qu'un seul adulte sur un total de 40 individus vivants.

Lorsque après les bains dans l'acide sulfurique concentré, il n'y eut en incubateur aucune immersion dans l'eau, les résultats furent comme suit (Tableau XXXI): trois minutes d'immersion nous donnent une moyenne de 12.2% d'émergence, ceci toutefois, n'est basé que sur 48 individus vivants et ne représente donc que six adultes. Après deux minutes dans l'acide sulfurique, l'émergence fut de 41.7% aussi sur un total de 48 individus, alors qu'après une minute d'immersion avec 75 individus vivants à la fin de l'expérience, l'émergence monta à 56%.

Bien que le petit nombre d'expériences rendit impossible l'analyse des résultats à l'aide des méthodes statistiques, il est donc fort probable que les immersions dans l'acide sulfurique concentré provoquent une reprise des activités plus considérable que tout autre facteur mis à l'épreuve. Le taux de 56% d'émergence que nous avons eu avec le traitement *i* est à peu près le double du développement obtenu au moyen d'immersions dans des solutions d'acide sulfurique à pH 3 qui, jusqu'ici, s'étaient avérées les plus efficaces. Il est vrai que lorsque les cocons furent en contact avec l'acide sulfurique concentré, la mortalité fut plus élevée que pour tout autre traitement, mais, encore une fois, ceci peut être attribué en partie à la manipulation. De plus, les résultats du Tableau XXXI nous portent à croire qu'un contact de quelques secondes seulement avec l'acide sulfurique activerait une reprise de développement plus considérable et moins de mortalité, car, à mesure que la durée des immersions diminue (de trois à une minute), le pourcentage de mortalité diminue parallèlement alors que l'émergence augmente.

Enfin, on peut conclure que de courtes immersions dans l'acide sulfurique concentré facilitent grandement la rupture de la diapause. Mais leur influence est plutôt le résultat de chocs provoqués à la fin de l'hibernation, qu'à la dessiccation qu'elles occasionnent au début, car dans de tels cas, il n'y a point ou très peu d'émergence.

Influence du milieu sur la rapidité de nymphose

Nous venons de voir que dans certains milieux la larve offre peu de résistance, et que par le fait même le taux de mortalité est plus élevé. Dans d'autres milieux, au contraire, les larves se nymphosent en grand nombre, mais on observe des différences appréciables dans chaque milieu quant à la vitesse de la rupture de la diapause.

La date d'émergence de chaque adulte ayant été notée, j'ai pu, après avoir groupé ensemble les lots ayant reçu des traitements semblables, établir la distribution de l'émergence pour chacun de ces traitements (Tableaux XXXII et XXXIII). Au moyen des méthodes statistiques, j'ai pu ensuite analyser ces distributions, les comparer et déterminer l'influence de différents milieux sur la rapidité de la rupture de la diapause chez *Diprion polytomum*.

TABLEAU XXXII

ÉMERGENCE DE *Diprion polytomum* APRÈS DIFFÉRENTS TRAITEMENTS

(Saison 1938-1939)

	Température d'hivernation		Température de nymphose		
	(1) 32°F.	(2) 32°F.	(3) 65°F.	(4) 75°F.	(5) 80°F.
Nombre d'individus éclos	266	93	34	146	91
Date moyenne d'éclosion	34.87	36.52	53.56	35.2	29.6
E.S.	12.3	11.0	11.25	9.3	10.1
E.S.M	0.75	1.14	1.93	0.77	1.06

TABLEAU XXXIII

ÉMERGENCE DE *Diprion polytomum* APRÈS DIFFÉRENTS TRAITEMENTS

(Saison 1940-1941)

	Hibernation en jours		Température d'hibernation		Humidité relative de nymphose		Variations brusques de température	
	(1) 84	(2) 112	(3) 45°F.	(4) 34°F.	(5) 75%	(6) 56%	(7) E	(8) F
Nombre d'individus éclos	83	92	13	31	23	19	38	24
Date moyenne d'éclosion	22.55	21.42	32.53	18.87	19.69	20.37	16.98	16.62
E.S.	5.07	6.42	9.45	2.06	8.22	8.67	2.42	4.32
E.S.M	0.551	0.64	2.63	0.37	1.72	1.99	0.39	0.88
	Immersions dans l'eau							
	(9) à 34°F.		(10) à 75°F.		(11) 2 hrs		(12) 3 hrs	
Nombre d'individus éclos	50		34		78		29	
Date moyenne d'éclosion	22.86		28.59		25.03		26.88	
E.S.	11.172		8.52		7.74		8.142	
E.S.M	1.58		1.46		0.87		1.39	
	Immersions dans solutions acides						(16) Immersions dans H ₂ SO ₄ conc.	
	H ₂ SO ₄				(15) Glycocolle à pH 5			
	(13) pH 5		(14) pH 3					
Nombre d'individus éclos	38		33		20		49	
Date moyenne d'éclosion	22.19		22.91		25.05		28.41	
E.S.	8.07		3.24		9.36		4.26	
E.S.M	1.29		0.56		2.09		0.60	

Le Tableau XXXII est basé sur les émergences de 1938-1939, alors que le Tableau XXXIII contient les résultats de mes expériences de 1940. Il aurait été intéressant de comparer les résultats de certains traitements rapportés dans ces deux tableaux; par exemple la rapidité d'émergence dans un milieu sec (Tableau XXXII) et dans un milieu humide (Tableau XXXIII). La chose fut impossible parce que la différence alors aurait pu être due non pas aux facteurs mis en cause, mais à des variations occasionnées par suite du fait que le matériel employé avait été récolté en deux années différentes.

TABLEAU XXXIV

ANALYSE STATISTIQUE SUR L'INFLUENCE DU TRAITEMENT
SUR LA RAPIDITÉ DE LA NYMPHOSE

(Voir Tableau XXXII)

Traitements comparés	Différence	E.S. _d
4 et 5	1.65	1.36
6 et 7	18.36**	2.08
7 et 8	5.60**	1.31

** Dépasse le niveau significatif de 1%.

TABLEAU XXXV

ANALYSE STATISTIQUE SUR L'INFLUENCE DU TRAITEMENT SUR LA RAPIDITÉ DE LA NYMPHOSE

(Voir Tableau XXXIII)

Traitements comparés	Différence	E.S. _d	Traitements comparés	Différence	E.S. _d
1 et 2	1.13	0.84	9 et 10	5.73*	2.14
3 et 4	16.66**	2.66	11 et 12	1.85	1.64
5 et 6	0.68	2.63	13 et 14	1.72	1.41
5 et 7	2.71	1.76	13 et 15	2.86	2.46
5 et 9	3.17	2.32	13 et 16	6.22**	1.43
7 et 8	0.36	0.93	15 et 16	3.36	2.18

* Dépasse le niveau significatif de 5%.

** Dépasse le niveau significatif de 1%.

De l'interprétation de ces distributions de l'émergence analysées aux Tableaux XXXIV et XXXV on peut déduire que:

1. La rapidité de nymphose est indépendante de la température d'hibernation, si celle-ci se produit à des températures voisines du point de congélation, ou inférieure à celui-ci, soit de 32° à 15°F. Si l'hibernation a lieu à des températures relativement élevées, par exemple 45°F., la reprise des activités est alors beaucoup plus lente; la moyenne d'émergence est alors de 32.5 jours, contre 18.9 jours à 34°F.

2. La rupture de la diapause n'est pas plus rapide après 12 semaines d'hibernation qu'après 16 semaines.

3. La vitesse de développement est en rapport direct avec la température de nymphose, augmentant avec celle-ci, jusqu'à ce que la limite vitale supérieure soit atteinte. Comme on pouvait s'y attendre la métamorphose est beaucoup plus lente à 65°F. Mais même entre les températures de 75° et 80°F., on constate un retard significatif de 5.6 jours à 75°F.

4. Les changements brusques des températures d'hibernation à celles de nymphose n'ont aucune influence sur la vitesse du développement.

5. L'humidité relative de l'incubateur lorsqu'elle varie entre 85 et 56% ne semble avoir aucune influence sur la vitesse de la reprise du métabolisme.

6. En général, les immersions dans l'eau favorisent un plus grand développement, mais dans certains cas, elles retardent la réactivation. Après les immersions durant l'hibernation seulement, les larves reprennent leur évolution avec un retard de faible amplitude qui, d'ailleurs, est sans importance comme l'indiquent les traitements 5 et 9, inclus dans le Tableau XXXV. Au contraire, lorsque les immersions ont lieu durant la nymphose, le retard est alors plus considérable et significatif, tel que le montrent les traitements 9 et 10, du Tableau XXXV.

7. Après des immersions de trois heures par semaine dans l'eau, l'émergence présente un retard de deux jours sur les immersions de deux heures par semaine, mais cette différence est négligeable.

8. Le pH n'a aucune influence sur la rapidité de nymphose. Après des immersions dans des solutions d'acide sulfurique à pH 3, la métamorphose survient presque en même temps; les dates moyennes d'émergence étant de 22.2 et de 22.9 jours. Ces chiffres nous montrent aussi que la réactivation est aussi rapide après les immersions dans l'acide sulfurique que dans l'eau, dans ce dernier cas, l'émergence moyenne étant de 22.9 jours.

9. On a vu que les solutions de glycolle à pH 5 n'augmentent pas la mortalité de façon notable. Elles n'influencent pas non plus la rapidité de la réactivation. A un pH de 5, la solution n'est pas assez concentrée pour retarder la rupture de la diapause et la plupart des adultes apparaissent après 25 jours soit trois jours plus tard qu'après les immersions dans l'acide sulfurique à pH 5, ce qui n'est pas significatif.

10. Après les immersions d'une et deux minutes dans l'acide sulfurique concentré à la fin de l'hibernation, les larves reprennent leur évolution avec un retard de six jours sur les immersions dans l'acide sulfurique à pH 5, et d'environ neuf jours sur les individus n'ayant subi aucune immersion. La reprise du développement est donc beaucoup plus lente après les chocs provoqués par l'acide sulfurique concentré.

Discussion et conclusions

Les Tenthredes faisant l'objet de la présente étude exhibent des diapauses de durées très variables. Chez *Diprion polytomum*, espèce sur laquelle a porté la plus grande partie de mes observations, la larve peut demeurer à

l'état de vie latente dans son cocon pendant des périodes variant de 10 jours à 7 ans. Des irrégularités aussi considérables dans la durée de la diapause ne peuvent s'expliquer simplement par l'action de facteurs ou agents n'agissant sur les individus qu'au cours de leur séjour dans leur cocon. Si tel était le cas, il ne serait pas après tout très difficile de mettre ces facteurs en évidence, et de briser ces longs sommeils, alors qu'au contraire dans bien des cas la chose est impossible.

Apparemment, pour résoudre la question de la diapause chez les Tenthredes, et plus particulièrement chez la mouche à scie européenne de l'épinette, il faudrait suivre les sujets en expérience avant même leur naissance, commencer dès l'éclosion des parents, connaître le nombre de jours et les conditions dans lesquelles la mère a vécu avant la ponte. Il faudrait aussi déterminer quelles sont les conditions optima pour l'incubation et le développement larvaire, et faire l'élevage des sujets dans ces conditions. Alors seulement nous pourrions espérer d'éliminer complètement la diapause. Puis, pour en déterminer les causes, il nous faudrait varier ces conditions idéales de milieu en commençant par un facteur à la fois, puis ensuite avec plusieurs en même temps. En connaissant les causes de la diapause, il nous serait alors plus facile de trouver les moyens pour y mettre fin. En un mot, il nous faudrait répéter avec *D. polytomum* les expériences exécutées par Cousin (30) avec *Lucilla sericata*. Ceci représenterait un travail de longue durée, mais dont les résultats seraient des plus intéressants. Nous ne perdons pas l'espoir de l'entreprendre sous peu. Le point faible du travail de Cousin provient du fait qu'elle a utilisé dans ses expériences, des espèces polyvoltines dont la diapause normalement n'est pas très prononcée, ni de longue durée et beaucoup plus facile à rompre que pour des espèces univoltines à longues diapauses, comme c'est le cas pour *Diprion*. C'est pourquoi, certains auteurs ont mis en doute certaines des conclusions de Cousin, car ils ne veulent pas considérer comme diapause un simple ralentissement dans le métabolisme, ralentissement cessant facilement lors du retour aux conditions normales. Mais si les expériences de Cousin étaient répétées avec un insecte comme *Diprion*, et que les résultats de cet auteur puissent être confirmés, comme je crois que ce serait le cas, il me semble que ceci serait suffisant pour prouver le bien-fondé de sa théorie.

Tout en faisant certaines restrictions pour les raisons ci-haut mentionnées, il n'est pas douteux que quel que soit le milieu dans lequel s'est effectué le développement embryonnaire ou larvaire, certains facteurs ont une action générale bien définie sur la rupture de la diapause. Ce fut le principal objet du présent travail de déterminer, au moyen d'études expérimentales, quelles sont les conditions qui, à l'automne, durant l'hiver et au printemps, peuvent augmenter ou diminuer la reprise du métabolisme chez les Tenthredes.

Les résultats essentiels de mes expériences et leur application dans leur milieu naturel me permettent de préciser les points suivants:

1. La différence observée dans le taux de mortalité après des hibernations à des températures variant de 45° à 15°F. est tout à fait insignifiante. *Diprion*,

de même que *Pristiphora*, résiste aussi bien à 15° qu'à 45°F. Cependant un stage assez long à 15°F. empêche ultérieurement la reprise des activités lorsque les conditions redeviennent favorables à la nymphose et, de plus, diminue la résistance des individus quand ceux-ci sont exposés à 75°F. et 85% R.H. Il semble donc que si, tard à l'automne, avant que le sol soit recouvert d'une couche de neige assez épaisse, la température du sol descend aux environs de 15°F. et s'y maintient assez longtemps, il peut en résulter une réduction dans le pourcentage d'émergence l'année suivante, et une mortalité anormale au début de l'été suivant. D'après Cousin (30), il ne faut pas toujours attribuer cette persistance de la diapause à des troubles causés directement par le froid, mais à la déshydratation qui résulte d'une chute thermique progressive. Dans plusieurs cas, la rupture de ces diapauses est alors possible par une exposition à une température chaude et *humide*. Quant au taux de développement après l'hibernation à 45° et 34°F., il est aussi considérable dans un cas comme dans l'autre, mais après une hibernation à 45°F., la réactivation est plus lente qu'après une hibernation à 34°F.

2. D'après Balch (7) quelques larves pourraient survivre à des hibernations à -1°F., alors que toutes mourraient à -20°F. Malheureusement, cet auteur n'indique pas la durée d'exposition nécessaire pour provoquer la mortalité à ces deux températures. Dans mes expériences aucune des espèces étudiées n'a pu vivre très longtemps à 0°F. *Diprion polytomum* n'a pas résisté plus de 10 semaines à cette température. Il en fut de même pour *Pristiphora erichsoni*, *Hylocoma pectoralis*, et *Neodiprion pinetum*. Il faut admettre, cependant, que des températures aussi basses se rencontrent rarement lorsque les larves se trouvent dans leur milieu naturel.

3. Lorsque la nymphose se fait dans un milieu sec, soit environ 56% R.H., la mortalité est plus élevée si l'hibernation s'effectue à des températures voisines ou inférieures au point de congélation, que si elle se produit à des températures bien supérieures à ce point.

4. Chez toutes les Tenthredes étudiées, après une durée minimum de 8 à 10 semaines à 45° et 34°F., il y a reprise du développement à des températures aussi basses que 60°F., et dans un milieu très sec. Mais, il va sans dire que le taux d'émergence est alors bien inférieur et moins rapide que celui que l'on obtient à des températures plus élevées. La rapidité de la reprise du métabolisme est en relation directe avec la température de nymphose, augmentant avec celle-ci.

5. Durant l'incubation, l'humidité relative plus que la température influence la résistance des individus. Ainsi une température de 85°F., associée à une atmosphère sèche, est nettement fatale aux Tenthredes; la plupart meurent en moins d'un mois. Tout au contraire, si l'humidité relative est élevée, les larves résistent mieux à cette même température qu'à la température optimum de 75°F., associée à une atmosphère sèche. D'un autre côté, lorsqu'il s'agit de la rupture de la diapause la température semble plus importante que l'humidité relative. A 85°F., même en milieu humide, la

larve reste en diapause, alors qu'à 75°F. et 85% R.H., la réactivation est aussi intense et aussi rapide qu'à la même température avec seulement 56% R.H.

6. Dans un milieu très sec, 20 à 30% R.H., le pourcentage d'adultes mourant dans leur cocon peut atteindre jusqu'à 50%, tout simplement parce que celui-ci est trop dur et sec pour que les adultes puissent couper leur trou de sortie. Il faut dire, cependant, que dans la nature de telles conditions hygrométriques doivent se rencontrer rarement.

7. A 90°F. et 20 à 25% R.H., aucune Tenthrede ne peut demeurer vivante dans son cocon plus d'une semaine. Après ce stage, les larves sont devenues brunes et dures, présentant la même apparence que celles dont les cocons durant l'été sont directement exposés aux rayons du soleil ou protégés seulement par une légère couche de mousse ou d'humus. On observe cet état de chose durant l'été dans les jeunes peuplements originant d'un feu ou d'un terrain déjà cultivé où le sol est très dur et où la couche d'humus est à peu près inexistante; les cocons se trouvant alors presque à la surface du sol. Dans d'autres peuplements d'épinette, on rencontre quelquefois des éclaircies constituées par des rochers recouverts d'une mince couche de matière végétale, sous laquelle nombre de larves cherchent refuge pour y tisser leur cocon. Durant l'été, la température sur ces rochers peut dépasser de beaucoup 90°F., et les individus qui ne sont pas éclos de bonne heure à l'été sont généralement tués plus tard par l'action de la chaleur.

8. Si l'on ne considère que les facteurs température et humidité relative, nous aurons la mortalité minimum et le maximum de développement après une hibernation aux environs du point de congélation et une nymphose à 75° ou 80°F., associée à une humidité relative de 85% ou plus.

9. Plusieurs auteurs notamment Roubaud (92), Parker (68), Cousin (30), et Balachowsky (6), ont noté l'influence de chocs thermiques sur la rupture de la diapause. Les expériences que j'ai faites à ce sujet sur *Diprion polytomum* permettent de distinguer deux séries de résultats. D'abord les variations brusques de température au début ou à la fin de l'hibernation ne réduisent pas la vitalité de *Diprion* lors de la période de nymphose. Ensuite les chocs thermiques au début de l'hibernation provoquent des diapauses plus difficiles à rompre. Au contraire, les mêmes chocs thermiques, s'ils ont lieu à la fin de l'hibernation entraînent une réactivation bien supérieure à celle obtenue après des changements graduels de température. Ces chocs, cependant, n'accélèrent pas la reprise du métabolisme. Si on interprète ces résultats, on peut estimer qu'à l'automne des changements subits et à grandes amplitudes dans la température pourraient réduire le pourcentage d'émergence l'année suivante, alors qu'au printemps, les mêmes changements subits amèneraient la nymphose d'un plus grand nombre de larves.

10. Les chocs provoqués par les immersions dans l'acide sulfurique concentré ont un effet analogue aux chocs thermiques, mais encore plus accentué que celui causé par ces derniers. Lorsque les immersions dans l'acide se font au début de l'hibernation, il n'y a point ou très peu d'émergence. Il doit alors se produire durant l'hibernation une forte dessiccation rendant la rupture

de la diapause très difficile, c'est-à-dire qu'ici l'acide sulfurique jouerait le même rôle que les froids excessifs. Par contre, après des immersions d'une minute à la fin de l'hibernation, l'émergence est presque deux fois plus considérable qu'après des immersions régulières de deux heures par semaine dans des solutions aqueuses. Les immersions dans l'acide sulfurique concentré raniment donc un grand nombre de larves qui autrement resteraient en diapause, mais cette reprise du métabolisme se fait avec un retard de six jours sur les immersions dans les solutions aqueuses à pH 5 et de près de neuf jours sur les individus n'ayant subi aucun contact avec l'eau.

11. A des températures voisines du point de congélation, les larves en diapause peuvent vivre plus de trois semaines complètement submergées dans l'eau, tandis qu'à 75°F., la larve ne peut supporter beaucoup plus qu'une semaine d'immersion. Dans nos forêts la température dans la mousse et l'humus n'atteint à peu près jamais 75°F. Mais il est probable que durant l'été, un sol saturé d'eau pendant plusieurs jours est fatal à un bon nombre de larves dans leur cocon, sans causer toutefois une mortalité de 100%.

12. Apparemment il y aurait plus de mortalité et moins d'émergence, après des immersions fréquentes mais de courtes durées, qu'après des immersions plus longues mais moins rapprochées. Cependant les résultats de ces expériences ne sont pas très concluants parce que dans le premier cas le taux de mortalité plus élevé peut, en partie, être attribuable à la manipulation.

13. Les immersions de deux ou trois heures par semaine durant l'hibernation et la nymphose entraînent la mort d'un plus grand nombre d'individus que si ces bains ne se produisent que durant l'hibernation. Il semble, de plus, que ce soit le contact de l'eau durant la nymphose qui soit particulièrement défavorable, la mortalité étant presque aussi élevée si les immersions n'ont lieu qu'à 75°F., que si elles se sont produites durant le séjour de la larve à 34° et à 75°F. Si l'on considère maintenant le développement, on remarque que lorsque les immersions dans l'eau ne se produisent que durant la nymphose, le taux d'émergence est inférieur à celui obtenu après des bains à 34°F. seulement, ou encore à 34° et 75°F. Dans ces deux derniers cas, le taux de développement ne diffère pas sensiblement. Ce serait donc les immersions durant l'hibernation qui activeraient surtout la reprise du développement. Babcock (3 et 4) lors de ses expériences sur la pyrale du maïs (*Pyrausta nubilalis*) remarqua que cet insecte est très sensible au contact de l'eau durant l'hibernation. S'il y a alors dessiccation, il en résulte un retard dans la pupaison ou diapause prolongée. Peut-être est-ce la même chose avec *Diprion polytomum*. Chose certaine c'est qu'après des immersions à 34°F., le métabolisme est beaucoup plus intense lors de l'exposition à 75°F. que si les individus n'ont eu aucun contact avec l'eau durant l'hibernation.

14. L'acidité du sol n'affecte en aucune façon le bien-être des larves dans leur cocon. Au contraire, elle active le métabolisme et à mesure que le pH diminue, le développement augmente. Les immersions dans les solutions d'acide sulfurique à pH 3 durant l'hibernation et la nymphose provoquent une réactivation plus grande que les immersions dans l'eau pure. D'après ces

résultats il ne semble pas y avoir de doute que dans les sols très acides le taux d'émergence devrait être plus considérable. Sautet (104) avait lui aussi constaté que certains composés chimiques amènent une réactivation rapide des larves en diapause d'*Anopheles bifurcatus*. Dans ses expériences ce rôle déclanchant était joué par des oxydants: eau de javel et permanganate de potassium, tandis que dans la nature, dit-il, "C'est l'oxygène provenant de la fonction chlorophyllienne des plantes aquatiques qui agit comme oxydant."

15. Les immersions dans le glyocolle sont néfastes aux larves dans leur cocon. Même durant l'hibernation leur influence se fait déjà sentir et lorsque les immersions se poursuivent durant la nymphose le pourcentage de mortalité devient alors considérablement plus élevé qu'après les immersions dans l'eau ou les solutions d'acide sulfurique. De plus le glyocolle inhibe considérablement la réactivation et lorsqu'il y a nymphose, le taux de mortalité à l'état de pupe ou d'adulte non éclos est plus élevé que dans tout autre milieu. Le glyocolle semble donc toujours toxique aux larves de *Diprion* dans leur cocon, et si dans les sols forestiers, il peut y avoir des concentrations de glyocolle ou même d'acides aminés en général aussi grandes que celles employées dans nos solutions aqueuses, on devrait s'attendre à un taux de mortalité plus élevé et à des diapauses plus longues.

16. Les immersions dans l'eau ou des solutions acides réduisent la durée de la diapause d'un mois. Dans le cas d'immersions durant l'hibernation et la nymphose dans des solutions d'acide sulfurique à pH 3 par exemple, nous avons presque autant d'émergence après huit semaines d'hibernation qu'il y en a après 12 semaines lorsque les cocons ne sont pas venus en contact avec des solutions aqueuses. Cependant, il reste toujours, même dans les lots ayant subi des immersions, que le nombre de larves qui se nymphosent est plus grand après 16 qu'après 12 semaines d'hibernation.

Chez des espèces ayant normalement des diapauses de plusieurs mois, voire même de plusieurs années, et cela depuis nombre de générations, on peut difficilement s'attendre qu'il soit possible de briser ces diapauses après un séjour de courte durée au froid. La chose devient d'autant moins probable lorsque les sujets ont vécu dans des milieux variables, non contrôlés et souvent défavorables, comme c'est le cas lorsqu'ils proviennent de leur milieu naturel. Il n'y a pas de doute alors que le facteur temps est important. Et même si les conditions de nymphose sont idéales, il ne peut y avoir métamorphose à moins que le sujet n'ait d'abord séjourné à de basses températures pour des périodes dont la durée est liée aux conditions dans lesquelles s'est effectué le développement embryonnaire et larvaire.

Parce que des traitements qui se sont montrés efficaces pour d'autres insectes n'ont pu mettre fin au sommeil hivernal de *D. polytomum* après trois ou quatre mois, nous ne sommes donc pas justifiés de conclure que chez cette espèce la diapause est héréditaire. Ces résultats ne prouvent qu'une chose: c'est que la reprise des activités n'est pas influencée, autant qu'on pourrait le croire, par les conditions prévalant, soit durant l'hiver, soit au printemps, lors de la métamorphose.

Si l'on compare les conditions climatiques dans les régions d'une ou plusieurs générations, l'on constate plusieurs faits venant à l'appui de cette hypothèse. Suivant Balch (7), le pourcentage de larves qui se transforment en adultes chaque année est toujours plus petit à mesure que l'on se dirige vers le nord. Dans la Gaspésie, la moyenne d'éclosion est de près de 20%, dans le centre du Nouveau-Brunswick elle est d'environ 70%, tandis qu'au sud de la Nouvelle-Angleterre, elle est encore beaucoup plus élevée. Serait-ce les conditions existant durant l'hiver ou le printemps qui pourraient être responsables des variations extérieures? Apparemment non, car durant tout son cycle vital c'est lorsque la larve est dans son cocon que les conditions de milieu se ressemblent le plus, indépendamment des régions ou des années. Ainsi, durant l'hiver, que ce soit dans la Gaspésie ou la Nouvelle-Angleterre, la température du sol sous la neige est à peu près la même. De plus, dans les peuplements d'épinette, où se trouve *D. polytomum*, certains sols peuvent être plus saturés les uns que les autres, mais au printemps, durant la période de nymphose, l'humidité en général est partout très élevée. Ce qui change le plus suivant les régions et les années, c'est la température de l'humus forestier après l'hibernation. Mais là encore, il est peu probable que les différences de température soient suffisamment élevées pour permettre d'expliquer les variations considérables de développement.

Dans les régions à une ou deux générations, ce n'est pas tant durant l'hibernation et la nymphose comme durant le développement embryonnaire et larvaire que l'on constate de grandes différences dans les conditions de milieu. Ainsi, dans certaines parties du comté de Rimouski et de la Gaspésie, il n'y a pas eu en 1937 et en 1938, un seul mois de l'année, où la température à 4 pieds du sol n'a pas descendu à 32°F. Il est peu probable que dans le sud du Nouveau-Brunswick et dans la Nouvelle-Angleterre l'on puisse observer en juillet et août de telles chutes thermiques aussi nettement défavorables aux œufs et aux larves et qui pourraient être suffisantes pour provoquer des diapauses de deux ou trois ans. En juin, alors que l'éclosion bat son plein, il arrive souvent dans la Gaspésie et sur la côte nord du St-Laurent que nous ayons une suite de journées froides qui empêcheront toute activité chez les femelles récemment écloses. Il en résulte un stage prolongé des œufs dans l'organisme maternel qui, d'après Roubaud, occasionnerait une espèce d'intoxication se traduisant plus tard par de longues diapauses.

De l'ensemble des faits précités, il semble que si l'on veut avoir une explication des longues diapauses de *Diprion polytomum* dans la partie nord de son habitat, nos études devraient être concentrées beaucoup plus sur les facteurs agissant au cours du développement embryonnaire et larvaire, que sur les conditions de milieu lorsque la larve est dans son cocon, c'est-à-dire durant l'hibernation et la nymphose. De telles études ne peuvent être faites dans un milieu extérieur fluctuant. Elles doivent être exécutées en laboratoire, afin d'obtenir des stocks homogènes, élevés dans des conditions optimales. De plus ces élevages doivent se poursuivre durant plusieurs générations, car même si la diapause n'est pas héréditaire, il n'en reste pas moins vrai qu'il

y a là, un rythme acquis depuis très longtemps et, comme l'a fait remarquer Cousin au sujet d'autres insectes, ce rythme peut fort bien ne pas disparaître complètement dès la première génération.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

IV. INFLUENCE OF FOOD AND DIAPAUSE UPON REPRODUCTIVE CAPACITY^{1,2}

By M. L. PREBBLE³

Abstract

Methods of sampling, determination of reproductive capacity, and analysis of data are described. Various physical measurements are positively correlated with reproductive capacity, but regression equations are unsatisfactory for estimation of fecundity outside of the population in which the relationships have been determined, due to variability in the degree of joint variation of size and fecundity under different feeding conditions. Field populations developed on white spruce are more fecund than those developed on black spruce; reductions of 30% or more may result from periodic food shortage.

Reproductive capacity of females emerging over a period of three to five years in each of 20 populations, failed to show any consistent trend in relation to the diapause period. From this, and also from the slight reduction in conymphal dry weight over extended intervals at favourable temperature, it is concluded that the destruction of conymphal reserves during diapause proceeds very slowly and has no practical effect upon fecundity of females issuing after prolonged diapause.

The reproductive capacity of an insect may be influenced by quantity and quality of food during larval development, and by food, water, copulation, viability of introduced sperm, longevity, and population density, among factors, affecting the adults (3, 5, 6, 7, 8, 11, pp. 391-394). The problem of determining the influence of factors during larval development may therefore be far from simple in species dependent upon a variety of factors during adult life. The problem in *Gilpinia polytoma* (Hartig) is less complicated because of several features: (1) absence of feeding after the fifth moult; (2) the parthenogenetic mode of reproduction; (3) the apparent lack of influence of environmental fluctuations during imaginal life upon ovarian development; and (4) the limited reproductive capacity which reduces the labour of counts.

Repeated tests have disclosed no consistent relationship between the following pairs of variables: (1) number of ova and percentage deposited; (2) adult longevity and number of ova; and (3) adult longevity and percentage of ova deposited. Females emerging after prolonged diapause are as vigorous as individuals without diapause. Percentage of hatch, determined in eight years in central Gaspé and two years in New Brunswick, has been uniformly

¹ Manuscript received in original form March 29, 1941, and as revised, July 25, 1941. This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Papers I and II of this series appeared in the October issue of the *Canadian Journal of Research*; Paper III in the November issue.

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high, nearly always over 90%. For these several reasons, the influence of food and diapause upon reproductive capacity of the spruce sawfly may be restricted to a study of the number of oocytes capable of being developed to maturity, all environmental effects during adult life being extraneous to the problem. Although the investigations were concerned chiefly with the effects of diapause, larval food, as will appear shortly, cannot be ignored in comparative tests.

The basic consideration is that reserves laid up in the eonymph must both sustain the insect during diapause and subsequent development, and also serve for the nourishment of oocytes in the maturing insect. Metabolic demands during prolonged diapause, if sufficiently large, might therefore be expected to result in reduced reproductive capacity.

Methods of Determination

The individual reproductive capacity of approximately 9000 females was determined over a period of years. Some females were placed singly in small cages in the field or laboratory, and the number of deposited eggs and retained oocytes determined. The much simpler method of counting oocytes in newly emerged females, considered capable of being developed to mature ova, was used in most instances. The defence of results thus obtained rests on an argument involving description of ovaries at adult emergence, development of oocytes during adult life, and a comparison of statistics obtained by the two methods.

Description of Ovaries

Each ovary consists typically of 15 ovarioles of the polytrophic type, the greenish-white oocytes* being readily distinguishable from the mottled dark and white nurse cell follicles. Though Smith (10) found four to five small differentiated oocytes in sections of pronymphal ovarioles, the present author has never found so many as a typical number either in newly emerged females, or in others at death, in which oviposition had been prevented. The average for field and laboratory reared females is between one and two oocytes per ovariole; occasionally three occur in a few ovarioles, and in one rare instance a female developed 102 ova, an average of 3.4 per ovariole. Apparently two or three of the young oocytes recognized by Smith in pronymphal ovarioles are arrested in their development and possibly absorbed before yolk formation.

The oocyte nearest the oviduct is usually mature at adult emergence, and the accompanying nurse cells are exhausted. Oocytes toward the germarium are smaller with larger nurse cells. Occasionally adults emerge precociously with all oocytes very small and the abdomen distended with fat body. Sterile females, with no development of the ovaries, were rare in this study, and were not attributable to poor food conditions, since they occurred at a frequency of 11 : 442 in an incubator reared pure line as compared with a ratio of 9 : 8100 in field material, much of which matured under severe conditions of host defoliation. Though the ovarioles within one ovary may

* Yellow in fixed material.

be variably developed, there is usually a remarkably close similarity in the production of the two ovaries of a pair.

Development of Oocytes

Mature ova measure about 2.0 by 0.3 mm. Most of the smaller oocytes which at adult emergence have a clearly recognizable yolk vary from one-quarter to three-quarters the size of ova. Some, however, are only about 0.2 mm. long, yet with sufficient yolk to distinguish them from the nurse cells. The presence of yolk, and not the size, was used as a criterion to establish which of the small oocytes should be included in the counts.

The development during adult life of oocytes which were small at emergence has been demonstrated by analysis of samples at different times. Immature oocytes constituted from 15 to 30% of the total capacity in newly emerged females in different populations, while at death of females in equivalent samples they constituted 2 to 5% of the total capacity. It is shown later that there was no essential difference in the total capacity of females dissected at emergence and at death, hence the reduction in immature oocytes proves that many of them were brought to maturity during adult longevity. This was entirely independent of oviposition.

There was no correlation between the percentage of immature oocytes at adult emergence and the duration of diapause, or between the percentage of immature oocytes and total capacity. It follows that high capacity and low capacity females were not so called merely by virtue of incidentally different stages of ovarian development at time of emergence. This is most clearly shown by the data in Table I, in which the females in different sample series from several populations are divided into classes of reproductive capacity representing the full range of variation. The proportion of immature oocytes was relatively constant for the different classes within each series and was much reduced, as already indicated, in series of females dissected at death. The relative constancy of the proportion of immature oocytes at emergence indicates a balance between the stored reserves and the number of oocytes that undergo development to and beyond the stage of yolk formation. This is a fortunate circumstance, since if there were a partial development of many oocytes prior to emergence, with subsequent absorption during adult life, counts made at emergence would be very inaccurate as a measure of reproductive capacity.

Comparative Results by Two Methods

Statistics on the average reproductive capacity of eight populations, as determined from series of females taken at random for dissection at emergence, dissection after oviposition, or dissection at death when oviposition was prevented, are summarized in Table II.

The mean reproductive capacity (\bar{x}) as determined in the oviposition and longevity series was not significantly different from that based on the emergence series in eight of the nine comparisons. In the other comparison (Experiment 302), in which the sample sizes were large, the difference though small was

TABLE I

THE RELATIVE CONSTANCY OF IMMATURE OOCYTES THROUGHOUT THE ENTIRE RANGE OF REPRODUCTIVE CAPACITY, FOR SERIES OF FEMALES FROM DIFFERENT POPULATIONS. *N* IS THE NUMBER OF FEMALES FALLING WITHIN THE VARIOUS CLASSES OF REPRODUCTIVE CAPACITY; THE PERCENTAGE VALUE IS THE RELATION OF IMMATURE OOCYTES TO THE TOTAL NUMBER OF OOCYTES

Expt. No.	Series*	Class limits of reproductive capacity																Entire series	
		0-25		26-30		31-35		36-40		41-45		46-50		51-55		56-60		N	%
		N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
123 (1937)	Em.	—	—	8	24.3	18	20.1	23	25.6	25	23.6	14	27.1	12	23.4	—	—	100	24.2
	Ov.	—	—	3	2.4	8	2.6	12	3.5	11	3.2	8	3.9	7	5.8	—	—	49	3.8
	Lon.	—	—	7	4.7	6	6.5	6	6.4	4	2.4	6	5.9	14	6.4	—	—	43	5.8
154 (1938)	Em.	24	26.6	29	27.8	62	22.1	59	22.9	52	25.1	24	25.5	17	21.2	—	—	267	24.0
	Ov.	5	4.3	10	3.5	17	3.6	16	4.7	11	3.6	3	2.8	3	4.7	—	—	65	3.9
302 (1938)	Em.	—	—	44	31.1	29	27.4	63	23.9	84	24.6	114	23.4	97	24.3	96	21.3	529	24.0
	Ov.	—	—	19	1.8	34	1.4	36	1.7	59	1.6	55	3.3	33	2.2	27	2.0	263	2.2

* Em. = emergence series; Ov. = oviposition series; Lon. = longevity series.

TABLE II

COMPARISON OF MEAN REPRODUCTIVE CAPACITY IN DIFFERENT SERIES OF FEMALES TAKEN AT RANDOM FROM A COMMON SOURCE

Expt. No.	Series	Number of females	\bar{x}	S.E. \bar{x}	Mean difference*	S.E. (diff.)	Significance
96 (1935)	Emergence Oviposition	75 50	51.92 50.38	0.93 1.17	1.54	1.49	None
96 (1936)	Emergence Oviposition	100 14	44.57 41.21	1.04 1.94	3.36	2.20	None
106 (1936)	Emergence Oviposition	100 62	47.84 46.69	0.81 1.28	1.15	1.52	None
115 (1936)	Emergence Oviposition	100 9	36.83 38.67	0.80 3.40	-1.84	3.49	None
98 (1937)	Emergence Oviposition	200 22	36.24 34.36	0.59 1.34	1.88	1.46	None
123 (1937)	Emergence Oviposition Longevity**	100 49 43	40.83 41.61 42.84	0.83 1.08 1.83	-0.78 -2.01	1.36 2.01	None None
302 (1938)	Emergence Oviposition	853 263	45.91 43.67	0.37 0.59	2.24	0.70	Good
154 (1938)	Emergence Oviposition	268 65	37.09 35.95	0.53 1.07	1.14	1.19	None

* The mean of the emergence series is the basis in all comparisons.

** Oviposition was prevented in the longevity series.

statistically significant. From this, considering also that in the other comparisons there was a majority of cases in which the determination based on the emergence series was the higher, there is some evidence of absorption of a few small oocytes during adult life, and consequently, of a corresponding positive error in determinations of reproductive capacity based on dissections of females at emergence. However, the discrepancy was small and the values obtained were evidently close approximations of true reproductive capacity. Inaccuracies inherent in the technique affect in a similar way practically all the determinations used in analysis of the influence of food and diapause, and therefore cancel out in the comparisons.

Methods of Analysis

Nature of Frequency Distributions

The nature of the frequency distributions of females according to their reproductive capacity requires description for two reasons: (1) normality or near normality of the distributions is a prerequisite of the usual techniques of analysis; (2) only by being assured of the adequacy of these techniques to the data in hand is one apt to appreciate the causes operative in obscuring relationship between diapause and reproductive capacity.

Much speculation was aroused when the possibility of various modal points in the population, suggesting the existence of strains differing in reproductive capacity, was indicated by graphic analysis of some of the earlier sample series of females. To reach a judgment as to the significance of the apparent modal points, three lines of investigation were carried on, none of them being a direct attack (which could only be attained by a program of selective line rearings beyond the possibilities of this project) but in the aggregate leading to a fairly certain conclusion.

The first phase of the investigation consisted of drawing off, from a known normal population of 1000 cardboard squares, samples of 50, 100, and 200 units, each unit being replaced in the population after the draw. Fairly

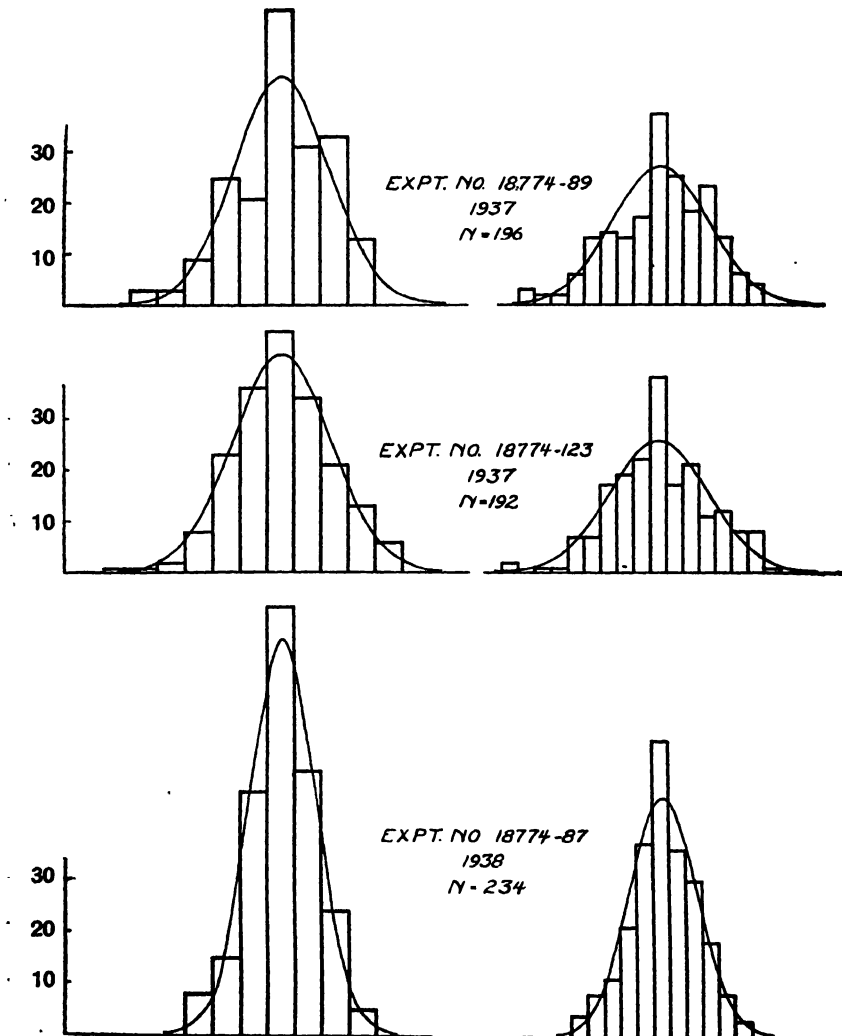


FIG. 1a

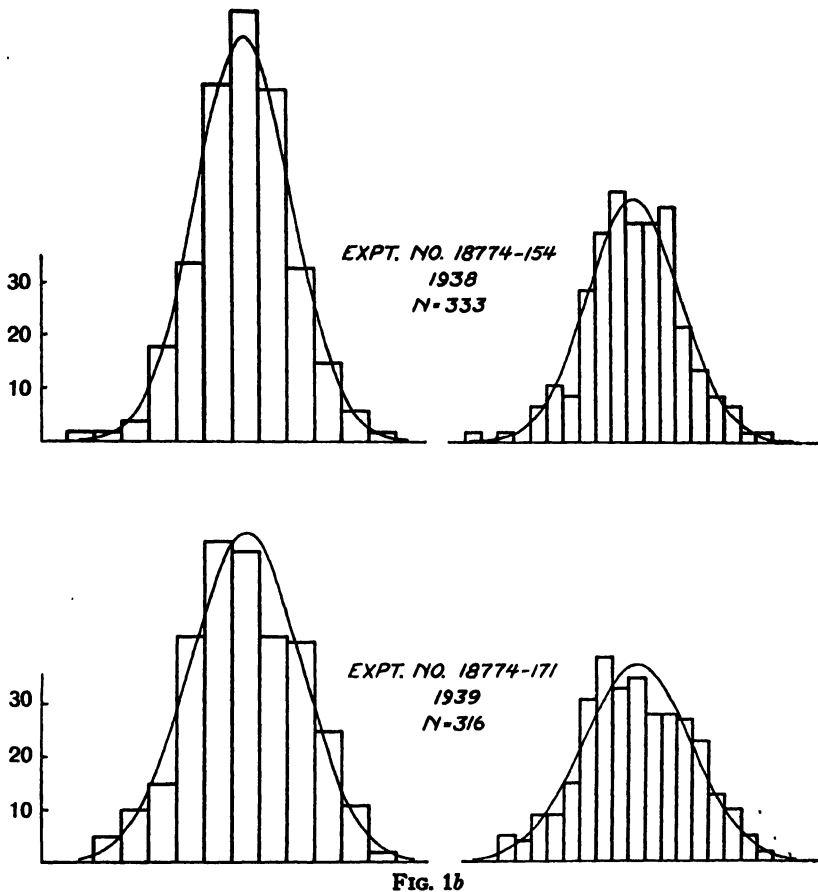


FIG. 1. Histograms and the theoretical normal distributions for samples of spruce sawfly females classified by the number of developing oocytes in the ovaries at emergence. Curves at the left are based on groupings in classes of five oocytes, those at the right on groupings in classes of three oocytes: a, samples of about 200 females; b, samples of over 300 females.

large departures from the population distribution occurred due to chance alone. Apparent modal points, having of course no real existence, were not uncommon in histograms based on samples of 50 and 100 units grouped in classes of three and five units, and occurred also in samples of 200 units. This showed conclusively that even with moderately large samples one must not expect distribution curves free of irregularity due to purely chance influences.

The later series of sawfly females were made as large as reasonably possible. The histograms for five series are illustrated in Figs. 1a and 1b; those at the left are based on groupings in classes of five oocytes, and those on the right, in classes of three oocytes, and the theoretical normal distributions are indicated by the smooth curves. The normality of the five distributions illustrated, and of seven others in which the sample size was moderately large, has been tested statistically by the Chi square test (Table III). The method

TABLE III

CHI SQUARE TESTS OF THE NORMALITY OF FREQUENCY DISTRIBUTIONS OF FEMALES CHARACTERIZED BY NUMBER OF OOCYTES. *P* INDICATES THE PROBABILITY THAT THE OBSERVED DISTRIBUTION CONFORMS TO THE HYPOTHESIS OF NORMALITY

Expt. No.	Number of females	Class interval, 3			Class interval, 5		
		Chi square	D.f.	<i>P</i>	Chi square	D.f.	<i>P</i>
25 (pure line)*	347	24.71	10	.01—	18.02	6	.01—
89 (1937)	196	15.41	10	.12	16.59	4	.01—
89 (1938)	345	17.86	10	.06	16.39	5	.01—
89 (all years)	662	22.39	13	.05	19.59	7	.01—
87 (1938)	233	7.19	6	.30	2.06	3	.56
87 (all years)	464	29.19	9	.01—	20.31	5	.01—
106 (1936)	162	6.56	9	.68	2.37	4	.67
98 (1937)	222	10.45	8	.24	5.41	4	.25
123 (1937)	192	12.91	10	.23	1.88	5	.86
154 (1938)	333	10.28	10	.42	3.83	6	.70
171 (1939)	316	9.76	12	.64	7.32	6	.29
13 (1939)*	233	14.74	10	.25	4.60	4	.34

New Brunswick material; all other lots were Gaspé material.

need not be described here and it is only necessary to remark that the tests were very rigorous since three degrees of freedom were subtracted, because of the use of N , \bar{x} , and $S.E.$ in the calculation of expected frequencies.

Five of the distributions based on groupings in classes of five oocytes, where degrees of freedom were necessarily reduced in number, had significant departures from normality; on the basis of groupings in classes of three oocytes, only two distributions had significant departures. The failure of several distributions to conform to the hypothesis of normality was due to moderate skewness, but since the means of moderately skewed distributions tend toward normality, these may be analysed in the usual way.

The final phase of the investigation was the analysis of reproductive capacity of females in a pure line, all descended parthenogenetically from a single individual in incubator rearings at constant conditions of food and temperature. The distribution was not free of the apparent modal points, and had a significant departure from the corresponding normal distribution (P less than .01) due to positive skewness. These results are instructive, since they prove that significant departures are not to be identified with heterogeneity in the population due to strains differing in reproductive capacity.

These considerations led to the conclusion that the apparent modal points in several of the distributions were merely artifacts due to chance, and that the analysis of data by procedures drawn up for normal populations was fully justified.

Method of Sampling

The method of making up the samples needs to be considered. In some of the experimental populations, very large numbers of females emerged

TABLE IV
OOCYTE COUNTS OF FEMALES DISSECTED AT EMERGENCE, IN SUCCESSIVE SUBSAMPLES OF FEMALES DURING THE EXTENDED EMERGENCE PERIOD

Expt. No.		Successive subsamples						Entire series
		1	2	3	4	5	6	
29 (1935)	Period N \bar{x} $S.E.\bar{x}$	July 10 - 14 29 36.07 0.95	July 15 - 18 19 35.84 2.10	July 19 - 21 16 41.06 1.25	July 22 - 25 26 42.54 1.84	—	—	90 38.79 0.84
96 (1936)	Period N \bar{x} $S.E.\bar{x}$	July 6 - 7 20 43.90 2.09	July 8 - 9 25 44.56 2.32	July 10 - 12 17 46.94 2.31	July 13 - 15 18 41.39 2.53	July 16 - 22 20 44.10 2.36	—	100 44.57 1.04
106 (1936)	Period N \bar{x} $S.E.\bar{x}$	July 6 - 7 15 49.87 2.16	July 8 23 49.65 1.62	July 9 - 10 25 45.56 1.39	July 11 - 14 21 47.33 1.93	July 16 - 20 16 47.56 2.29	—	100 47.84 0.81
89 (1937)	Period N \bar{x} $S.E.\bar{x}$	July 1 - 6 40 39.20 1.39	July 8 - 11 38 36.42 1.31	July 12 - 16 42 38.64 1.50	July 17 - 19 32 44.12 1.22	July 20 - 30 44 39.48 1.28	—	196 39.41 0.63
98 (1937)	Period N \bar{x} $S.E.\bar{x}$	July 1 - 5 29 36.83 1.30	July 6 - 8 38 39.79 1.13	July 9 - 11 35 33.69 1.07	July 12 - 14 35 33.20 1.62	July 16 - 17 36 38.33 1.37	July 18 - 20 27 35.04 1.88	200 36.24 0.59
154 (1938)	Period N \bar{x} $S.E.\bar{x}$	June 17 - July 5 24 38.25 1.74	July 6 - 8 50 39.02 1.21	July 9 97 36.41 0.85	July 10 - 12 40 38.18 1.28	July 13 - 15 29 34.76 1.87	July 16 - Aug. 7 28 35.89 1.66	268 37.09 0.53
302 (incubator)	Period N \bar{x} $S.E.\bar{x}$	Feb. 26 - Mar. 11 127 46.79 0.89	Mar. 12 - 17 103 46.14 1.07	Mar. 18 - 22 100 48.26 1.02	Mar. 23 - April 1 102 46.58 1.14	April 2 - 23 99 42.22 1.25	—	531 46.05 0.48

during three or four weeks. The earlier practice in such cases was to take the first 100 to 200 females as the sample. If high capacity and low capacity individuals tended to emerge at different times samples restricted to one portion of the emergence period might be seriously biased. In order to make an analysis of this point, the females in seven series were broken down into subsamples according to time of emergence (Table IV) and the subsample means critically compared (Table V).

Significant differences between the highest and lowest subsample means occurred in five of the seven series, but significant differences between the general sample mean (best estimate of population mean) and means of component subsamples occurred in only four of 36 comparisons. There was no evidence of a tendency for the more fecund and less fecund individuals to

TABLE V

ANALYSIS OF DIFFERENCES BETWEEN THE MEANS OF VARIOUS SUBSAMPLES OF FEMALES DISSECTED AT EMERGENCE. MARGINAL DIFFERENCES HAVE BEEN TESTED BY THE CALCULATION OF "t" AS DESCRIBED IN TEXT

Expt. No.	Highest and lowest subsample means			General mean and highest subsample mean			General mean and lowest subsample mean			Number of subsamples not significantly different from general mean	Position in series	
	Diff.	S.E.	Signif.	Diff.	S.E.	Signif.	Diff.	S.E.	Signif.		(a), of lot with highest mean	(b), of lot with lowest mean
29	6.70	2.79	Yes	3.75	2.02	Yes*	2.95	2.26	No	3 of 4	Latest	2nd of 4
96	5.55	3.43	No	2.37	2.53	No	3.18	2.74	No	5 of 5	3rd of 5	4th of 5
106	4.31	2.57	No	2.03	2.31	No	2.28	1.61	No	5 of 5	Earliest	3rd of 5
89	7.70	1.79	Yes	4.71	1.38	Yes	2.99	1.46	No*	4 of 5	4th of 5	2nd of 5
98	6.59	1.97	Yes	3.55	1.28	Yes	3.04	1.72	No	5 of 6	2nd of 6	4th of 6
154	4.26	2.23	Yes*	1.93	1.32	No	2.33	1.94	No	6 of 6	2nd of 6	5th of 6
302	6.04	1.61	Yes	2.21	1.13	No	3.83	1.34	Yes	4 of 5	3rd of 5	Latest

* The conclusion as to significance, contrary to the relationship of the values shown, is based on the more critical "t" test.

emerge at restricted intervals in the emergence period (Table V, last two columns). Neither was there any significant difference in the degree of variability* among individuals emerging at different times. In conclusion, sampling restricted to a limited portion of the emergence period would only occasionally introduce a bias, and this possibility was eliminated in most of the experimental series that were studied either by including all emerging individuals in the sample, or by taking representatives over the entire emergence period.

Significance of Differences

In the calculation of the standard error of a mean difference between two series, the formula,

$$S.E. (\bar{x}_1 - \bar{x}_2) = \sqrt{(S.E. \bar{x}_1)^2 + (S.E. \bar{x}_2)^2}$$

* The coefficients of variability for the various subsamples have not been included in the already extensive tabulation, but are easily derivable from the statistics shown.

was used as a first approximation (all standard errors of differences shown in the tables were so derived), in the test of significance of the mean difference; the latter having to be at least twice the standard error to be adjudged significant. This test, however, lacks precision, especially where samples being compared are not large and differ in size. Therefore all marginal cases were tested by a more rigorous test in which the two individual variances are pooled to provide the best estimate of the difference variance and standard error. The significance of the critical "t" value derived from the mean difference and its standard error also depends upon sample size. The method is fully described in standard texts (2, pp. 40-42) and needs no further comment here.

Food and Reproductive Capacity

Larvae of *Lymantria dispar* Linn. which were starved on alternate days during the final instar produced pupae 48% lighter in weight, and female adults 53% lower in egg production, than controls (4). The production of viable eggs by *Ephestia kuhniella* Zeller was 14 to 32% less with white flour as the larval food, compared with wholemeal, and 44% less when the larval ration of wholemeal was reduced by 50% from the optimal ration of 0.15 gm. per larva (7).

Reproductive capacity in the spruce sawfly is significantly correlated with various physical measurements. The results of some preliminary analyses in which the possibility of using some simple measurements as an index of reproductive capacity was investigated, are shown in Table VI. Gross

TABLE VI

CORRELATION BETWEEN REPRODUCTIVE CAPACITY AND PHYSICAL MEASUREMENTS IN VARIOUS SAWFLY POPULATIONS, AND THE REGRESSION EQUATIONS FOR ESTIMATING REPRODUCTIVE CAPACITY (Y) FROM THE MEASUREMENT (X)

Measurement	Source of material	Correlation coefficient	Regression equation
Head width*	New Brunswick field material, 123 females	$+ .667 \pm .050$	$Y = -71.088 + 3.8534 X$
Head width	Gaspé field material, 853 females	$+ .526 \pm .025$	$Y = -130.528 + 5.481 X$
Head width	New Brunswick pure line in incubator, 347 females	$+ .652 \pm .031$	$Y = -147.810 + 6.3254 X$
Gross weight, cocoon and contents (cg.)	New Brunswick pure line in incubator, 347 females	$+ .758 \pm .023$	$Y = 2.968 + 7.861 X$
Cocoon length (mm.)	New Brunswick pure line in incubator, 347 females	$+ .652 \pm .031$	$Y = -61.712 + 13.231 X$

* Head width of adult in arbitrary units, $50X = 3.7084 \text{ mm.}$

weight, just after cocoon spinning, was most highly correlated with reproductive capacity ($r = .758$); cocoon length and head width of the adult were slightly less valuable as indices of reproductive capacity. It is most instructive to note, that while a fair estimate of reproductive capacity within a given population could be obtained from the corresponding regression equation, the

latter was unsatisfactory for prediction outside of the population on which it was founded. This is evidenced by the dissimilarity of the three regression equations, reproductive capacity on head width, for different populations. This means that while size and fecundity vary in the same direction under the influence of food and other factors during larval development, the degree of their joint variation is not precisely the same in different populations. This recalls the experience of Schedl (9) who was in error by 43% of the actual fecundity in estimates of a Danzig population of *Diprion pini* Linn., based on the regression established for a population of the insect near the Dutch frontier.

White spruce as the larval host typically yields larger and more fecund females than black spruce (Table VII). The mean reproductive capacity

TABLE VII

COMPARISON OF THE MEAN REPRODUCTIVE CAPACITY OF FIELD COLLECTED MATERIAL FROM WHITE SPRUCE AND BLACK SPRUCE. ALL LOTS WERE FROM CENTRAL GASPÉ AND THE FEMALES EMERGENTS OF THE FIRST SEASON AFTER COCOON COLLECTION, EXCEPT WHERE OTHERWISE STATED

	Expt. No.	N	\bar{x}	S.E. \bar{x}	Remarks
Host White spruce 1935	7	14	45.43	1.69	Emergents after 5-year diapause Emergents after 4-year diapause
	31	61	36.97	0.87	
	85	18	62.05	1.33	
	89	83	48.96	1.06	
	96	125	51.30	0.73	
1936	104	100	50.80	0.92	
	106	162	47.40	0.70	
1937	300	105	37.60	0.97	
	301	69	45.83	1.03	
1938	137	15	57.93	2.15	
	143	18	57.00	1.57	
1939	167	70	60.03	1.19	Material from York Co., N.B.
	300 N	60	55.17	0.91	
Host Black spruce 1935	29	90	38.79	0.84	Emergents after 5-year diapause Emergents after 4-year diapause
	30	83	35.89	1.09	
	98	70	38.97	1.15	
1936	115	109	36.98	0.78	
1937	123	192	41.48	0.65	
	120	132	47.11	0.75	
1938	154	333	36.87	0.47	Material from Sunbury Co., N.B.
	1	122	53.37	0.57	
1939	165	122	45.66	0.81	Material from Sunbury Co., N.B.
	171	316	39.02	0.57	
	13	233	53.00	0.55	

of field populations on white spruce was occasionally over 60, frequently over 50, and lower values were due to unsatisfactory feeding conditions; on black spruce, a value of 50 to 55 indicated excellent feeding conditions, one less than 40, a scarcity of suitable foliage.

Experimental evidence of the effect upon fecundity of intermittent food shortage during late larval development was obtained by random division of field collected fourth instar larvae into two groups, one of which was continually supplied with foliage, and the other deprived of food for alternate intervals of two to three days. In three replications (Table VIII), the survivors of the second group suffered a reduction of 27 to 37% in reproductive capacity.

TABLE VIII

COMPARISON OF THE MEAN REPRODUCTIVE CAPACITY OF FEMALES ENSUING FROM LARVAE THAT WERE FED CONTINUALLY, AND FROM OTHERS INTERMITTENTLY STARVED DURING LATE LARVAL DEVELOPMENT. OTHER FACTORS WERE THE SAME FOR BOTH GROUPS, THE HOST WAS WHITE SPRUCE, AND THE REARINGS WERE CARRIED OUT IN CENTRAL GASPÉ

Replication	Basis	Larvae fed continually			Larvae intermit- tently starved			Reduction, %
		N	\bar{x}	<i>S.E.</i> \bar{x}	N	\bar{x}	<i>S.E.</i> \bar{x}	
1 (1934)	Emergents of first year	68	47.75	0.76	82	33.85	0.63	29
	Emergents of all years	199	43.68	0.44	117	31.87	0.59	27
2 (1935)	Emergents of first year	62	44.79	0.77	56	30.39	1.29	32
	Emergents of all years	124	43.67	0.55	126	27.71	0.88	37
3 (1935)	Emergents of first year	20	37.65	1.10	13	24.46	1.74	35
	Emergents of all years	28	37.21	0.84	72	26.10	0.84	30

Studies of successive generations of pure lines reared without diapause in the incubator have shown that there is not necessarily any correlation between fecundity of parent and offspring, since this is determined within the limits of normal variation by the feeding regimen.

Diapause and Reproductive Capacity

The effect of diapause upon reproductive capacity appears to have received little study. The sole reference known to the writer is that of Cousin (1), who, as a result of observations and one experiment in which 25 normal females produced 10,619 maggot offspring and 25 diapause females produced 7591, concluded that diapause is prejudicial to fecundity of *Lucilia sericata* Meig. To what extent the reduced productivity was influenced by vigour, longevity, mating, hatching, and chance, was not determined.

The reproductive capacity of four series of sawfly females which emerged after four to five years in diapause is shown in Table VII. Comparison of these values among themselves, and with the values of other series lacking prolonged diapause, shows the futility of attempting to trace a relationship

between diapause and fecundity in different populations where uncontrolled factors, chiefly food, are so variable.

The procedure followed from 1935 onwards consisted of determining reproductive capacity of females emerging in successive years from a common source, by which is meant a large population of field collected larvae or cocoons from a single host and restricted area. In order for the successive yearly means to truly represent the influence of diapause, three basic conditions must be satisfied. 1. The original population must be homogeneous to keep experimental error as small as possible; this was controlled by limiting each population to material from one host, one locality generally less than an acre in extent, and one time of collection; tests of normality of distributions described previously provide evidence of homogeneity. 2. There must be no tendency for selective mortality among more or less fecund members of the population during diapause, a prerequisite for which no direct proof was obtained, but which seems to be established by presumptive evidence; if there were such selective mortality, females emerging in successive years would tend definitely towards greater or less fecundity, and the absence of any such inclination in 20 populations may be taken to substantiate the prerequisite. 3. The females emerging each year must provide a representative cross section of the then existing population, more and less fecund members in proportion to their occurrence in the population. Consideration of this prerequisite is undertaken in a later paragraph.

The data for several series of females from each of 20 populations are summarized in Table IX, and the intrapopulation differences are analysed in Table X. In 19 comparisons between first and second year series, 14 dif-

TABLE IX

MEAN REPRODUCTIVE CAPACITY (\bar{x}) OF FEMALES EMERGING IN SUCCESSIVE YEARS FROM A COMMON SOURCE

Expt. No.		1935	1936	1937	1938	1939
85	N \bar{x} $S.E.\bar{x}$	18 62.05 1.33	3 50.33 6.64	23 50.70 1.32	8 52.00 1.91	—
87	N \bar{x} $S.E.\bar{x}$	72 52.51 0.99	4 44.50 3.28	154 47.14 0.61	233 48.36 0.40	—
89	N \bar{x} $S.E.\bar{x}$	83 48.96 1.06	33 34.36 2.05	196 39.41 0.63	345 37.54 0.43	—
96	N \bar{x} $S.E.\bar{x}$	125 51.30 0.73	114 44.16 0.95	—	—	—
98	N \bar{x} $S.E.\bar{x}$	70 38.97 1.15	98 29.58 1.04	222 36.05 0.55	—	—

TABLE IX—*Concluded*

MEAN REPRODUCTIVE CAPACITY (\bar{x}) OF FEMALES EMERGING IN SUCCESSIVE YEARS FROM A COMMON SOURCE—*Concluded*

Expt. No.		1935	1936	1937	1938	1939
234	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	$\begin{matrix} 68 \\ 47.75 \\ 0.76 \end{matrix}$	$\begin{matrix} 3 \\ 41.67 \\ 0.68 \end{matrix}$	$\begin{matrix} 62 \\ 42.50 \\ 0.65 \end{matrix}$	$\begin{matrix} 64 \\ 40.91 \\ 0.57 \end{matrix}$	—
235	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	$\begin{matrix} 82 \\ 33.85 \\ 0.63 \end{matrix}$	$\begin{matrix} 5 \\ 24.40 \\ 3.15 \end{matrix}$	$\begin{matrix} 30 \\ 27.70 \\ 0.92 \end{matrix}$	—	—
100	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 20 \\ 50.40 \\ 2.19 \end{matrix}$	—	$\begin{matrix} 20 \\ 43.35 \\ 1.75 \end{matrix}$	$\begin{matrix} 53 \\ 48.70 \\ 1.00 \end{matrix}$
102	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 26 \\ 49.23 \\ 1.78 \end{matrix}$	$\begin{matrix} 6 \\ 51.67 \\ 4.04 \end{matrix}$	$\begin{matrix} 91 \\ 47.08 \\ 0.94 \end{matrix}$	$\begin{matrix} 18 \\ 45.56 \\ 2.22 \end{matrix}$
114	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 43 \\ 49.12 \\ 1.42 \end{matrix}$	$\begin{matrix} 61 \\ 43.89 \\ 0.94 \end{matrix}$	$\begin{matrix} 28 \\ 44.50 \\ 1.52 \end{matrix}$	—
115	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 109 \\ 36.98 \\ 0.78 \end{matrix}$	$\begin{matrix} 137 \\ 39.54 \\ 0.80 \end{matrix}$	$\begin{matrix} 124 \\ 35.42 \\ 0.74 \end{matrix}$	$\begin{matrix} 36 \\ 41.11 \\ 1.39 \end{matrix}$
257	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 62 \\ 44.79 \\ 0.77 \end{matrix}$	$\begin{matrix} 3 \\ 37.67 \\ 5.81 \end{matrix}$	$\begin{matrix} 28 \\ 41.93 \\ 0.89 \end{matrix}$	$\begin{matrix} 31 \\ 43.58 \\ 1.14 \end{matrix}$
258	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 56 \\ 30.39 \\ 1.29 \end{matrix}$	$\begin{matrix} 9 \\ 16.67 \\ 2.64 \end{matrix}$	$\begin{matrix} 30 \\ 25.10 \\ 1.33 \end{matrix}$	$\begin{matrix} 31 \\ 28.61 \\ 1.89 \end{matrix}$
260	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	$\begin{matrix} 13 \\ 24.46 \\ 1.74 \end{matrix}$	$\begin{matrix} 14 \\ 27.64 \\ 2.48 \end{matrix}$	$\begin{matrix} 34 \\ 25.06 \\ 1.16 \end{matrix}$	$\begin{matrix} 11 \\ 29.27 \\ 1.66 \end{matrix}$
118	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 79 \\ 45.19 \\ 1.09 \end{matrix}$	$\begin{matrix} 15 \\ 35.80 \\ 2.15 \end{matrix}$	$\begin{matrix} 13 \\ 40.08 \\ 1.64 \end{matrix}$
119	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 52 \\ 38.96 \\ 1.64 \end{matrix}$	$\begin{matrix} 28 \\ 41.61 \\ 1.98 \end{matrix}$	$\begin{matrix} 153 \\ 46.86 \\ 0.88 \end{matrix}$
120	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 132 \\ 47.11 \\ 0.75 \end{matrix}$	$\begin{matrix} 88 \\ 45.36 \\ 0.86 \end{matrix}$	$\begin{matrix} 42 \\ 44.38 \\ 1.33 \end{matrix}$
121	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 83 \\ 43.88 \\ 0.99 \end{matrix}$	$\begin{matrix} 5 \\ 45.20 \\ 3.86 \end{matrix}$	$\begin{matrix} 55 \\ 37.75 \\ 1.15 \end{matrix}$
123	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 192 \\ 41.48 \\ 0.65 \end{matrix}$	$\begin{matrix} 74 \\ 36.62 \\ 0.91 \end{matrix}$	$\begin{matrix} 103 \\ 41.20 \\ 0.88 \end{matrix}$
124	$\begin{matrix} N \\ \bar{x} \\ S.E.\bar{x} \end{matrix}$	—	—	$\begin{matrix} 35 \\ 44.26 \\ 1.37 \end{matrix}$	$\begin{matrix} 18 \\ 38.39 \\ 1.42 \end{matrix}$	$\begin{matrix} 32 \\ 41.66 \\ 1.19 \end{matrix}$

TABLE X

ANALYSIS OF DIFFERENCES BETWEEN MEAN REPRODUCTIVE CAPACITY OF FEMALES EMERGING IN DIFFERENT YEARS FROM A COMMON SOURCE. THE FIRST YEAR, ETC., REPRESENTS THE FIRST SEASON OF EMERGENCE AND WAS NOT NECESSARILY THE SAME CALENDAR YEAR IN DIFFERENT POPULATIONS. DIFFERENCES ARE POSITIVE REPRESENTING AN APPARENT INCREASE, EXCEPT WHERE NOTED TO THE CONTRARY

Expt. No.	1st to 2nd years			1st to 3rd years			2nd to 3rd years			3rd to 4th years		
	Diff.	S.E.	Signif.	Diff.	S.E.	Signif.	Diff.	S.E.	Signif.	Diff.	S.E.	Signif.
85	-11.72	6.77	No	-11.35	1.87	Yes	0.37	6.77	No	1.30	2.32	No
87	-8.01	3.43	No*	-5.37	1.16	Yes	2.64	3.34	No	1.22	0.73	No
89	-14.60	2.31	Yes	-9.55	1.23	Yes	5.05	2.15	Yes	-1.87	0.76	Yes
96	-7.14	1.20	Yes	—	—	—	—	—	—	—	—	—
98	-9.39	1.55	Yes	-2.92	1.28	Yes	6.47	1.18	Yes	—	—	—
234	-6.08	1.02	No*	-5.25	1.00	Yes	0.83	0.94	No	-1.59	0.86	No
235	-9.45	3.21	Yes	-6.16	1.12	Yes	3.30	3.28	No	—	—	—
100	—	—	—	-7.05	2.80	Yes	—	—	—	5.35	2.02	Yes
102	2.44	4.41	No	-2.15	2.01	No	-4.59	4.15	No	-1.52	2.41	No.
114	-5.23	1.70	Yes	-4.62	2.08	Yes	0.61	1.79	No	—	—	—
115	2.56	1.12	Yes	-1.56	1.08	No	-4.12	1.09	Yes	5.69	1.58	Yes
257	-7.12	5.86	No	-2.86	1.18	Yes	4.26	5.88	No	1.65	1.45	No
258	-13.72	2.94	Yes	-5.29	1.85	Yes	8.43	2.96	Yes	3.51	2.31	No
260	3.18	3.03	No	0.60	2.09	No	-2.58	2.74	No	4.21	2.03	No*
118	-9.39	2.41	Yes	-5.11	1.97	No*	4.28	2.70	No	—	—	—
119	2.65	2.57	No	7.90	1.86	Yes	5.25	2.17	Yes	—	—	—
120	-1.75	1.14	No	-2.73	1.53	No	-0.98	1.59	No	—	—	—
121	1.32	3.98	No	-6.13	1.52	Yes	-7.45	4.03	No	—	—	—
123	-4.86	1.12	Yes	-0.28	1.09	No	4.58	1.27	Yes	—	—	—
124	-5.87	1.98	Yes	-2.60	1.82	No	3.27	1.85	No	—	—	—

* Decision based on "t" test as described in text.

ferences are negative, representing a decrease in fecundity, of which five differences are statistically significant; of the five positive differences, one is significant. In comparisons between first and third year series, 17 differences are negative (11 significant) and two differences are positive (one significant). In comparisons between second and third year series, and between third and fourth year series, positive differences are more frequent than negative ones, and seven of the 20 positive differences are significant. In several of the populations, females of the fourth year had approximately the same, or greater, mean reproductive capacity than females of the first year. The data provide little concrete evidence that females emerging in successive years have a progressively reduced fecundity due to the metabolic demands upon onymphal reserves during diapause.

All steps in the procedures have been examined and found to be essentially sound, with the exception of the third prerequisite, viz., that each year's emergence must represent a true cross section of the then existing population. If this were true, and if metabolic demands during diapause were sufficiently great, then the distribution of females in different classes within the range of variation in reproductive capacity would be essentially the same from year to year, except for a gradual shifting toward the lower categories with prolongation of diapause. Comparisons of the distributions of females in

TABLE XI

DISTRIBUTION OF FEMALES IN DIFFERENT CLASSES WITHIN THE RANGE OF VARIATION IN REPRODUCTIVE CAPACITY, FOR EMERGENTS OF DIFFERENT YEARS FROM A COMMON SOURCE. THE VALUES SHOWN ARE THE PERCENTAGES OF THE TOTAL SEASONAL EMERGENCE FALLING WITHIN THE RESPECTIVE CLASSES

Expt. No.	Year	N	Class limits of reproductive capacity					
			0 - 30	31 - 35	36 - 40	41 - 45	46 - 50	51 -
98	1935	70	18.6	20.0	12.8	27.2	11.4	10.0
	1936	98	57.2	14.3	16.3	4.1	6.1	2.0
	1937	222	26.2	23.5	25.3	13.1	6.3	5.8
	All years	390	32.5	20.6	20.8	13.3	7.2	5.6
115	1936	109	22.0	22.9	24.8	15.6	9.2	5.5
	1937	137	14.6	14.6	24.8	19.0	13.1	13.9
	1938	124	24.2	26.6	23.4	13.7	8.9	3.2
	1939	36	11.1	13.9	13.9	33.3	11.1	16.7
	All years	406	19.2	20.5	23.4	17.7	10.6	8.6
120	1937	132	3.8	6.8	7.6	25.0	20.4	36.4
	1938	88	4.5	9.1	10.2	19.3	23.9	33.0
	1939	42	11.9	2.4	11.9	26.2	21.4	26.2
	All years	262	5.3	6.9	9.2	23.3	21.8	33.5
123	1937	192	9.4	16.7	21.3	20.8	14.6	17.2
	1938	74	23.0	17.6	27.0	21.6	6.8	4.0
	1939	103	11.7	13.6	18.4	21.4	22.3	12.6
	All years	369	12.7	16.0	21.7	21.2	15.2	13.2

successive years, for four populations with moderately large numbers, are shown in Table XI.

Only in one population, viz., 120, do the successive yearly distributions even moderately conform to the hypothesis. This, in fact, was the only one of the 20 populations studied over a period of years that showed a progressive reduction in reproductive capacity, though even here the differences were not significant.

The failure of the third prerequisite provides an explanation of the irregular fluctuations in annual mean reproductive capacity. Observed differences need be significant of nothing more than a departure in the distribution of seasonal emergents from the true population distribution. In fact, there is some evidence that the resumption of development may not affect at random the more and less fecund members of the population, but may be biased toward one or the other group according to wholly unknown factors. This is illustrated by the data in the accompanying synopsis, for various samples of a large cocoon population incubated under different conditions. The means of three samples were significantly different from the combined mean of all five (best estimate of the population mean), showing that even when difference in time was eliminated, the resumption of development was not purely at random among the members of the various samples, which incidentally were all large.

Conditions of incubation	Development, %	Number of females	\bar{x}	$S.E.\bar{x}$
1. Dec. 17: 74° F., 100% R.H.*	8-10	92	41.82	1.08
2. Feb. 7: Provided with contact water, then 74° F., 100% R.H.	60-95	531	46.05	0.48
3. Feb. 7: Dried two weeks, then contact water, and later 74° F., 100% R.H.	10	147	48.42	0.72
4. Feb. 7: Dried four weeks, then as in No. 3	7	74	46.55	1.20
5. Feb. 7: Dried six weeks, then as in No. 3	5	9	33.55	3.28
Combined results		853	45.91	0.37

* R.H. = relative humidity.

In conclusion, it would seem that prolonged diapause cannot entail a serious depletion of the eonymphal reserves, otherwise the ultimate effect upon reproductive capacity would not be masked by sources of error such as those described.

Changes in Weight During Diapause

In order to obtain more direct evidence of the rate of depletion of reserves, dry weight determinations** were made for samples of eonymphs in diapause in a number of experimental populations. Most of the samples consisted of 40 to 50 eonymphs. The results are briefly discussed in the following paragraphs.

a. Gaspé material, 1937-1938.

The average dry weight of eonymphs in diapause after 40 days at 75° F. was 1.96 cg., compared with an average of 1.98 cg. for eonymphs which, extracted from the cocoons at the start of the experiment, died within a few days. The difference is only 1.1 times its standard error and therefore not significant.

b. Gaspé material, 1938-1939.

The average dry weight of eonymphs after three months at 55° to 65° was 1.916 cg. (average of eight samples), and that of eonymphs killed at the start, 1.963 (average of 12 samples). The difference, 0.047 cg. with a standard error of 0.015, is statistically significant and represents a loss of about 2.4%.

** Fat constitutes about 35% of the dry weight.

c. New Brunswick material, 1938-1939.

Samples of cocoons were incubated at 74° F. Dry weight determinations were made at the start and after 10 days of incubation, 44 samples in all being analysed. The average weights were 2.19 cg. and 2.18 cg., the apparent loss (0.01 cg.) being less than its standard error (0.012) and without significance.

d. New Brunswick material, 1938-1939.

In another experiment with the same population as in *c*, dry weight determinations were made for various samples at intervals up to 18 days after incubation at 74° F. The accompanying synopsis is based on three complete replications involving 48 samples.

Period at 74°	Av. dry weight	Period at 74°	Av. dry weight
0	2.20	10	2.19
2	2.20	14	2.19
4	2.18	18	2.18
6	2.19		

In this series regression of dry weight on time, to a limit of 18 days, is without significance.

e. New Brunswick material, 1938-1939.

Dry weight determinations were made for successive samples from the same field population at intervals through practically a year, with results as follows.

Date	Av. dry weight	Date	Av. dry weight
Oct. 29, 1938	2.17	Mar. 7, 1939	2.26
Nov. 19	2.24	Mar. 21	2.21
Nov. 21	2.23	April 4	2.18
Dec. 6	2.21	April 7	2.19
Dec. 13	2.18	May 5	2.16
Dec. 27	2.20	May 13	2.18
Jan. 10, 1939	2.20	May 26	2.15
Feb. 6	2.18	Sept. 1	2.12
Feb. 21	2.16	Oct. 10	2.20
Feb. 22	2.22		

The regression of dry weight on time for this series indicates an apparent loss of about 2% during the year. The error of estimate is high, however, and it is informative to note that the final sample had a higher value than the first one.

With such slight weight changes during diapause it is not surprising that no clear trends in reproductive capacity with prolongation of diapause have been discovered.

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THE DIAPAUSE AND RELATED PHENOMENA IN *GILPINIA POLYTOMA* (HARTIG)

V. DIAPAUSE IN RELATION TO EPIDEMIOLOGY^{1,2}

BY M. L. PREBBLE³

Abstract

This final paper in the series on diapause in the spruce sawfly describes the direct and indirect influences of diapause on epidemiology in different parts of the distribution range in eastern North America. These relate to duration of diapause, degree of diapause in overwintered cocoons, and proportions of the population surviving to participate in the continuation of the infestation. The nature of infestations in different areas is described briefly.

Discussion of features of intraspecific differences, and a summary of conclusions derived from all five papers in the series, are also included.

The degree of diapause in overwintered cocoons, the number of years that the insect may remain in diapause, the ultimate survival, and the number of seasonal generations, are variable factors in different parts of the distribution range of the spruce sawfly in North America and greatly influence the course of infestations. Reproduction by parthenogenesis is highly favourable to the insect, but this factor, being uniform throughout the distribution range, does not enter into comparisons between localities.

Diapause in a One-Generation Area

Duration of Diapause

Sawfly emergence in central Gaspé has been recorded in successive years for over 200 lots of field collected cocoons, which contained a mixture of generations of preceding years, and of reared cocoons of a single season. Each lot was kept in a shallow flat of wood and wire screening under the moss from September to early June, and in a wooden container with screen bottom resting on the forest floor in a shaded location, during the summer. Results for representative lots are summarized in Table I.

Emergence from a lot was seldom complete in less than four years, and frequently extended into the fifth or sixth year. There was a small emergence in the seventh year after spinning of the cocoons in a few large lots in which mortality during diapause was very low. Prolonged diapause was equally typical of mixed field populations, lots of cocoons spun at the same time and of the progeny of single females, regardless of the parental diapause period.

¹ Manuscript received in original form March 29, 1941, and as revised, July 25, 1941. This paper is based on part of a thesis submitted to the Faculty of Graduate Studies and Research of McGill University, Montreal, Que., in April 1940, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

² Papers I and II of this series appeared in the October issue of the *Canadian Journal of Research*, Paper III in the November issue, and Paper IV in this issue.

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TABLE I

EMERGENCE IN SUCCESSIVE YEARS FROM REPRESENTATIVE LOTS OF GASPÉ COCOONS. THE LOTS CONSISTED OF FIELD COLLECTED COCOONS UNLESS OTHERWISE NOTED

Expt. No.	Time of collection	Original number of cocoons	Emergence in successive years									Total emergence, %
			1932	1933	1934	1935	1936	1937	1938	1939	1940	
13	June, 1932	866	104	10	338	92	5	—	—	—	—	63
14	June, 1932	497	76	65	146	27	—	—	—	—	—	63
30	July 5, 1932	444	40	16	74	110	22	—	—	—	—	59
31	July 4, 1932	388	49	38	50	65	4	1	—	—	—	53
42	June, 1933	3322	—	1548	302	244	81	—	—	—	—	65
45	June, 1933	1519	—	725	21	189	163	3	—	—	—	72
46	June, 1933	435	—	99	5	9	7	1	—	—	—	28
49*	Sept. 1-8, 1933	533	—	—	40	2	54	114	13	—	—	42
50*	Sept. 8-16, 1933	246	—	—	36	1	75	56	—	—	—	68
51*	Sept. 16-23, 1933	321	—	—	80	2	25	67	1	—	—	55
52*	Sept. 23-30, 1933	389	—	—	202	4	44	29	—	—	—	72
53*	Sept. 30-Oct. 7, 1933	273	—	—	186	0	5	1	—	—	—	70
63	June, 1934	2089	—	—	442	35	174	245	—	—	—	43
64	June, 1934	1840	—	—	410	33	192	309	10	—	—	52
65	June, 1934	1687	—	—	382	33	84	32	—	—	—	31
66	June, 1934	1696	—	—	387	8	188	65	2	—	—	38
81	June, 1934	494	—	—	8	1	74	244	21	—	—	70
85*	Aug. 20-Sept. 3, 1934	100	—	—	—	18	3	24	9	—	—	54
86*	Sept. 3-10, 1934	687	—	—	—	52	4	113	53	—	—	32
87*	Sept. 10-17, 1934	1303	—	—	—	75	4	188	238	—	—	39
88*	Sept. 17-24, 1934	1226	—	—	—	123	4	263	135	—	—	43
89*	Sept. 24-Oct. 1, 1934	1261	—	—	—	146	33	263	363	10	—	65
A**	1934	199	—	—	—	84	6	30	18	4	—	71
B**	1934	55	—	—	—	25	1	6	5	—	—	67
C**	1934	232	—	—	—	115	14	32	29	—	—	82
D**	1934	94	—	—	—	79	—	—	—	—	—	84
97	June, 1935	2259	—	—	—	506	284	434	3	—	—	54
98	June, 1935	3359	—	—	—	579	115	309	—	—	—	30
115	June, 1936	1367	—	—	—	—	364	142	125	42	6	50
116	June, 1936	1217	—	—	—	—	350	94	124	72	6	53
123	June, 1937	1072	—	—	—	—	—	520	77	104	10	66
125	June, 1937	857	—	—	—	—	—	364	104	152	0	†
126	June, 1937	659	—	—	—	—	—	246	47	78	0	56
152	June, 1938	712	—	—	—	—	—	—	160	17	52	†
153	June, 1938	616	—	—	—	—	—	—	112	15	21	†
154	June, 1938	1319	—	—	—	—	—	—	328	23	245	†

* Lots consisted of cocoons spun by field collected mature larvae, over intervals noted.

** Lots consisted of progeny of virgin females, reared in small cages. Parents of Lot A had a 1-year diapause period; of Lot B, a 2-year diapause period; of Lot C, a 3-year diapause period; of Lot D, a 4-year diapause period.

† Lots contained living cocoons in the fall of 1940, to yield further emergence in 1941 and later.

Reduced emergence in the second year, followed by an increase in the third or fourth year, was typical of the great majority of lots under study. The tendency was independent of the calendar year in which the lots were started, so that the "second year low" in many lots coincided with the "third or fourth year high" in others; hence the trend could not have been due to climatic variations in different seasons. The trend was also observed in a number of experiments in which the cocoons were left in the undisturbed forest floor in which they were spun, eliminating the possibility that the unusual distribution of seasonal emergence was in some obscure way brought about by the method of handling the experimental lots. No explanation can be offered for the phenomenon.

A picture of the average trend of yearly emergence in the Gaspé population is given in the following synopsis, based on 134 lots started in various years and in which emergence was completed by 1940.

Year	1	2	3	4	5	6	Total
Number of sawflies	16,190	2862	7812	6103	295	14	33,276
Percentage of total	48.65	8.60	23.48	18.34	0.88	0.04	99.99

The total emergence over all years was close to 50% of the original number of cocoons. About half of this emergence occurred in the first year, and most of the balance in the third and fourth years.

Proof that prolonged diapause is an entirely normal phenomenon in central Gaspé was obtained from quadrates of the undisturbed forest floor, which were isolated by means of screen cages permanently staked down with the sides banked with moss to prevent entry of larvae seeking a place to spin. The yearly emergence from cages (each two feet square) in the black spruce slope, where initial cocoon population was dense, follows:

	1932	1933	1934	1935	1936	1937	1938	1939
Three cages set down in June, 1932	19	72	20	12	10	4	2	—
Six cages set down in June, 1934	—	—	108	32	53	35	13	2

Variations in Diapause

Representative data on variations in the degree of diapause in central Gaspé, based on cocoon samples collected in the spring before emergence started, are summarized in Table II. The validity of the estimates may be recognized from the close agreement of results obtained by different methods.

TABLE II

VARIATIONS IN THE PERCENTAGE OF DIAPAUSE IN OVERWINTERED COCOONS IN DIFFERENT FOREST TYPES IN CENTRAL GASPÉ, 1932-1940. ALL LOTS CONSISTED OF COCOONS COLLECTED IN THE LATE SPRING OF THE YEAR FOR WHICH THE RECORD IS GIVEN

Year	Berry Mountain Brook						Brandy Brook			
	White spruce flat		Black spruce slope		Black spruce flat		Black spruce slope		Black spruce flat	
	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %	No. of cocoons	Diapause, %
1932	1736 1735*	95 96	1806 941*	88 89	268	91	—	—	—	—
1933	434	77	4841	53	—	—	—	—	—	—
1934	2499 9243†	77 74	6110	78	686	91	—	—	—	—
1935	2508 378*	86 82	2259 139*	78 73	—	—	3359 331*	83 88	—	—
1936	1414	88	1717	71	1142	69	2584	72	—	—
1937	33	76	219	53	512	67	2588	56	229	76
1938	522	92	419	81	2258	91	2647	77	2099	93
1939	511	78	526	74	1102 1070††	80 77	1450	72	941 7999† 1012††	83 82 78
1940	—	—	—	—	—	—	339 235* 461††	29 27 39	214* 467††	38 44

* Analyses of cocoons collected periodically during pronymphal and pupal development.

† Periodic analyses of samples from a single large collection in early June.

†† Cocoons left in wire flats in the moss, analysed in the autumn. All other lots were kept in wooden containers in the moss.

Diapause in the overwintered populations of different forests and years varied from about 30 to over 95%. There was only an approximate parallelism in the trend of the degree of diapause in contiguous areas from year to year, showing that other factors than climatic variations were involved. Studies already described have shown that temperature variations within the limits of occurrence in the Gaspé forest had no perceptible effect on diapause in the overwintered populations; and while moisture deficiency in late May and June in some instances notably increased the percentage of diapause, the extreme alternative, viz., an abundance* of moisture at all times, did not result in a considerable reduction in the phenomenon.

* Moisture from melting snow and rainfall is usually abundant due to the water-retaining capacity of the deep moss in the Gaspé forest. Rainfall usually exceeds three or four inches per month during the growing season. Moss samples taken periodically in four seasons had an average water content of 320% (dry weight basis), and the underlying humus of 350%. The values never fell below 40 and 100%, respectively, in the driest periods.

A fairly striking relationship between the time of larval maturity and the degree of emergence in the following season, later members of the population giving a significantly higher emergence than earlier members, occurred in several series in which the successive lots of larvae matured under progressively declining temperature conditions (cf. Lots 49 to 53, Table I). On the other hand, a progressive increase in emergence did not occur in other series in which the successive lots of larvae matured in the absence of a definite temperature decline (cf. Lots 85 to 89, Table I).

Another instance in which seasonal development was to a large extent conditioned in the previous autumn was noted in 1939 and 1940. An unusually large proportion (37%) of the overwintering population in the black spruce flat, Brandy Brook, consisted of very young to moderately developed pronymphs in the fall of 1939, a phenomenon which was not explained satisfactorily, but which nevertheless was largely contributory to the unusually low diapause (about 42%) in the same population in the 1940 season, since as explained elsewhere, individuals that enter the developmental season as pronymphs do not fail to develop.

Considering the peculiar trend of seasonal emergence in the experimental lots, it is logical to suppose that the degree of diapause in a natural population will be influenced by the relative importance of the component parts. A high proportion of two-year-old cocoons would presumably lower the general development very appreciably. The age composition of a natural population of sawfly cocoons is indeterminable, but definitely varies from year to year and reflects the relative success of the immediately preceding larval generation.

In conclusion, variations in the degree of diapause in central Gaspé cannot be explained satisfactorily on the basis of climatic variations. Much of the variability between populations of different stands and years is apparently due to the previous history of the populations, while the more or less uniformly high degree of diapause appears to be an inherent characteristic of the Gaspé population as a whole.

Epidemiology

The sawfly outbreak in central Gaspé was already in an advanced stage when discovered in 1930. In addition to advantages resulting from the mode of reproduction and absence of parasitism, the great extent of spruce forest provided an unusual opportunity for the development of a large and destructive population. As the studies of the bionomics continued it became evident that the course of the outbreak was largely influenced by the diapause phenomenon, which restricted the population of feeding larvae and exposed the dormant population to the continual operation of factors causing mortality.

The progress of the cocoon population in typical spruce stands was followed from year to year, and representative data for three stands appear in Table III. It should be noted that the cocoon shells are very durable and persist for many years, and that although the number of living cocoons varies from

TABLE III

COCOON POPULATION AND MORTALITY, SAMPLE PLOTS IN CENTRAL GASPÉ, COUNTS
MADE IN JUNE

Locality	Year	Square feet in sample	Cocoons per square foot						Total mortality, %
			Living	Emerged	Chewed	Insect-killed	Dead	Total	
Berry Mt. Brook, white spruce flat	1933	80	6.0	3.0	15.7	1.4	11.0	37.1	76
	1934	96	20.3	4.3	17.2	1.2	9.3	52.3	53
	1937	34	2.0	9.7	24.6	8.1	12.9	57.3	80
	1937	35*	0.9	9.2	24.3	9.1	12.9	56.4	82
Berry Mt. Brook, black spruce slope	1932	100	14.4	11.5	16.6	3.5	1.5	47.5	45
	1933	600	8.7	10.4	16.8	3.4	2.3	41.6	54
	1934	288	22.2	10.1	20.7	2.4	1.8	57.2	43
	1935	224	11.0	11.9	29.9	2.5	1.9	57.2	60
Brandy Brook, black spruce slope	1935	200	17.6	12.8	20.8	5.2	4.0	60.4	50
	1936	200	13.5	15.4	22.8	4.9	5.2	61.8	53
	1937	312	9.3	20.1	31.3	5.7	5.0	71.4	59
	1938	200	13.9	27.4	43.7	7.7	5.8	98.5	58
	1939	200	8.6	30.0	45.9	11.2	5.4	101.2	62
	1940	200	5.3	31.2	48.2	12.6	4.8	102.1	64

* Samples taken under trees that had been killed by defoliation in preceding years.

year to year, the total number cannot show a decrease except as influenced by sampling fluctuations.

The principal causes of mortality were shrews, mice, and squirrels, which made characteristic chewed openings in the cocoons, elaterid larvae, which made small circular or irregular openings, and physical factors, especially excessive moisture, which caused the death of the insect without immediate change in the cocoon, although the latter tend to collapse later. The total mortality in active infestations ranged from about 45 to 65% in well drained black spruce slopes, and from about 50 to 75% in valley-bottom stands of white spruce.

It is obvious that mortality values based on the total number of cocoons have to be interpreted according to the proportion of living cocoons in the population, and according to the season at which the counts are made. If, for purposes of establishing a standard of comparison, one might assume that living cocoons will subsequently come to the same fate as the empty cocoons (emerged and dead), then it is possible to calculate comparable statistics independent of the age of the infestation or the time of sampling in relation to seasonal development. The principal interest here is in the proportion of the population that has participated in continuation of the infestation, and the calculation is therefore the percentage relationship of emerged cocoons to all empty cocoons. The accompanying synopsis includes such statistics for the data in Table III.

Forest type and locality	Year	Survival, %
White spruce flat, Berry Mountain Brook	1933	10
	1934	13
	1937 (live trees only)	18
	1937 (dead trees only)	17
	Average	15
Black spruce slope, Berry Mountain Brook	1932	35
	1933	32
	1934	29
	1935	26
	1935	30
Brandy Brook	1936	33
	1937	32
	1938	32
	1939 (all trees)	32
	1939 (dead trees only)	35
	1940 (all trees)	32
	1940 (dead trees only)	33
	Average	32

The difference in survival between the two forest types is highly significant, reflecting the effect of poor drainage in the white spruce flat. The uniformity of the annual estimates, particularly for the black spruce slope where the most intensive sampling was done, substantiates the basic assumption of a fate for living cocoons similar to that of their predecessors, *provided of course that no new elements of control enter the complex*. This qualification is important in respect to results obtained in New Brunswick, though not so far applying to central Gaspé where no perceptible change in the control elements has occurred between 1932 and 1940.

It may be concluded that about 15% of the population in the white spruce flat, and about 32% in the black spruce slope, survived the period of dormancy to continue the infestation.

A valuable analysis of the role of diapause in contributing to rapid termination of an outbreak of *Diprion similis* Hartig in Poland is given by Hardy (3). Diapause in cocoons surviving the winter was almost 100%, and there was no addition to the population during the summer. Meanwhile disease and cocoon parasites flourished in the dormant population, quashing the outbreak in a single year.

The only instance of extremely high diapause in the spruce sawfly within our experience, viz., 98 to 99% at Parke Reserve in 1934, had no such catastrophic consequences as those reported by Hardy. The reduction in living cocoons per square foot during the year was as follows:

	May, 1934	May, 1935	Reduction, %
Samples under white spruce	27.7	18.8	32
Samples under black spruce	13.1	8.3	37

Emergence was about 12% in 1935, and since then the infestation has followed a normal course. Based on population records over a 5-year period, survival has averaged 23%.

Due to the high degree of diapause and comparatively low survival from the period of dormancy, infestations in a typical one-generation area are moderately slow in development but persistent, being protected by the reserve in diapause from the effects of very unfavourable conditions in a particular year. The only factor that has been noted as responsible for a steady decline in population of living cocoons in a one-generation area is the severe defoliation and death of the host. Current additions to the population may fail to compensate for current depletions (emergence and mortality) when the remaining old foliage is reduced to 10% or less of the normal complement of a healthy tree. At this stage the reduced population may still be denser in relation to food supply than during the stages of population increase, and there may even be a temporary increase during an exceptionally favourable year. An illustration of this occurs in the accompanying synopsis, showing cocoon density, adult emergence, and condition of the host trees in a black spruce slope in central Gaspé.

Year	Density per square foot		Condition of host trees		
	Living cocoons	Emerging females	Percentage dead*	Surviving trees**	
				Percentage of defoliation	Percentage of twig mortality
1935	17.6	3.0	2	75	31
1936	13.5	3.8	5	85	37
1937	9.3	4.1	9	95	64
1938	13.9	3.2	19	93	52
1939	8.6	2.4	29	87	51
1940	5.3	3.6	32	88	54

NOTE: Population counts made in June, condition of host trees checked in September. The season of 1937 was exceptionally favourable, reflected in the population of June 1938.

* Cumulative.

** Ocular estimate; the slight reduction in injury to the surviving trees in later years was partly due to elimination of most severely injured trees through mortality, and partly to errors in estimating.

The evidence obtained so far therefore indicates that without some new element of control†, the spruce sawfly in one-generation areas is likely to persist as a serious menace as long as there is host material to support it.

Diapause in a Two-Generation Area

The data on diapause in a two-generation area relate principally to south central New Brunswick. A few observations pertaining to the State of Maine are included for comparison.

† The liberation of exotic parasites is of course designed to introduce the needed element of control. Results in central Gaspé, to date, do not justify an expectation that the current infestation in that area will be significantly altered. In other areas, more recently and less severely infested, the outlook is more encouraging, due both to greater parasite effectiveness and to a virus disease which kills the developing larvae.

Duration of Diapause

The successive yearly emergence from a number of experimental lots of cocoons is shown in Table IV. Most of these lots were kept in wood-covered containers during the summer, and since it has more recently been shown that withholding moisture during the summer may result in a prolongation of diapause in New Brunswick material, it follows that the results are not entirely typical of the natural field populations. The error is on the conservative side, and therefore may be regarded as strengthening the significance of the difference between the diapause periods of populations in one-generation and two-generation areas.

TABLE IV

EMERGENCE IN SUCCESSIVE YEARS FROM LOTS OF NEW BRUNSWICK FIELD COLLECTED COCOONS

Locality	Time of collection	Original number of cocoons	Emergence in successive years					Total emergence, %
			1935	1936	1937	1938	1939	
Estey Bridge, York Co.	May, 1935	160	133	7	—	—	—	87
Estey Bridge, York Co.	May, 1936	101	—	60	26	—	—	85
Stanley, York Co.	Oct., 1935	342	—	204	53	33	—	85
South Tay, York Co.	Oct., 1935	280	—	120	87	50	—	92
South Tay, York Co.	May, 1936	125	—	74	41	—	—	92
Young's Brook, York Co.	June 4, 1936	167	—	41	69	34	—	86
Young's Brook, York Co.	May 6, 1937	414*	—	—	268	54	—	78
Young's Brook, York Co.	May 18, 1937	1420*	—	—	851	338	81	89
McNamee, Northumberland Co.	May, 1935	149	34	75	24	—	—	89
McNamee, Northumberland Co.	May, 1936	135	—	60	33	29	—	90
McNamee, Northumberland Co.	May, 1937	476	—	—	117	76	215	86
Canaan River, Queens Co.	Nov., 1936	1110*	—	—	673	128	—	72

* These lots were kept in screen covered cages during the summer. All others were kept in wood covered cages. See text.

The average distribution of seasonal emergence from the lots summarized in Table IV is as follows:

Year	1	2	3	Total
Number of sawflies	2635	987	466	4088
Percentage of total	64.5	24.1	11.4	100

The total emergence was 84% of the original number of cocoons, the range in individual lots, 72 to 92%.

Variations in Diapause

Data on variations in the percentage of diapause in different New Brunswick localities, based on samples collected in the spring of the year for which the record is given, appear in Table V.

TABLE V

VARIATIONS IN THE PERCENTAGE OF DIAPAUSE IN OVERWINTERED COCOONS IN DIFFERENT LOCALITIES IN NEW BRUNSWICK, 1935 TO 1940

Year	Locality	Number of cocoons	Diapause, %
1935	McNamee, Northumberland Co. Estey Bridge, York Co.	149	77
		160	17
1936	McNamee, Northumberland Co. Estey Bridge, York Co. South Tay, York Co. Young's Brook, York Co.	135	55
		101	41
		125	41
		167	75
1937	McNamee, Northumberland Co. Young's Brook, York Co.	476	75
		1834	39
1938	Millville, York Co. Acadia Expt. Sta., Sunbury Co.	3483	8
		148	29
1939	St. Leonard, Madawaska Co.	196	50
	McNamee, Northumberland Co.	540	19
	Blissfield, Northumberland Co.	2359	31
	Glencoe, York Co.	1789	14
	Zionville, York Co.	1008	13
	Cross Creek, York Co.	806	8
	Nashwaak, York Co.	2078	27
	English Settlement, York Co.	4675	11
	Acadia Expt. Sta., Sunbury Co.	865	23
1940	McNamee, Northumberland Co.	269 (Lot 1)	20
		1354 (Lot 2)	36
	Blissfield, Northumberland Co.	1536	10
	Nashwaak, York Co.	652	35
	Durham, York Co.	118	9
	Hampton, Kings Co.	2032	15
	Kingston, Kings Co.	1811	16

The values for the first two years are in all probability somewhat high, because not only was rainfall excluded, but partly developed individuals were not detected as the remaining cocoons, being required for subsequent emergence, were not opened in the autumn. As a generalization, from about 8 to 40% of the overwintered cocoons in south central New Brunswick (Kings, Sunbury, and York counties) remained in diapause throughout the season. Farther north, in Northumberland and Madawaska counties, the values ranged from 19 to about 70%.

Peirson and Nash (4) report that 35% of the overwintered cocoons in Aroostook County, Maine, remained in diapause during the 1939 season.

Epidemiology

The spruce sawfly was known to be distributed throughout south central New Brunswick in 1933, but only at a very low population level. Nowhere was there any noticeable defoliation. There was no apparent change in population density in several localities where plot studies were in progress during the following two or three years. Beginning in 1936 in some localities and in 1937 in others, there was a marked increase in population accompanied by defoliation injury which in some woodlands became severe within two years of the first sign of larval feeding.

TABLE VI

COCOON POPULATION AND MORTALITY, SAMPLE PLOTS IN NEW BRUNSWICK. AVERAGES TO NEAREST 0.1 EXCEPT WHERE VERY SMALL

Locality	Year	Square feet in sample	Cocoons per square foot						Total mortality, %
			Living	Emerged	Chewed	Insect-killed	Dead	Total	
Hanwell Road, York Co.	Oct., 1934	160	0.4	0.4	0.1	0.1	0.02	1.1	21
	Oct., 1935	160	0.1	0.7	0.3	0.3	0.05	1.5	46
Fredericton, York Co.	Oct., 1934	76	0.3	0.4	0.1	0.1	0	0.9	18
	Oct., 1935	72	0.2	0.8	0.2	0.2	0.04	1.4	30
McNamee, Northumberland Co.	Oct., 1934	32	1.0	0.2	0.2	0.5	0	1.8	36
	May, 1935	196	0.8	0.4	0.3	0.3	0.04	1.8	37
	May, 1936	196	0.7	0.5	0.7	0.4	0.1	2.3	50
	May, 1937	196	2.6	0.9	0.9	0.5	0.1	5.0	29
	May, 1938	196	5.8	1.2	1.4	1.4	0.1	9.9	29
Estey Bridge, York Co.	May, 1935	272	0.6	0.7	0.1	0.03	0.03	1.4	11
	May, 1936	280	0.4	1.1	0.3	0.05	0.03	1.8	20
Royal Road, York Co.	Oct., 1935	200	1.7	1.1	0.06	0.15	0.04	3.1	8
	Oct., 1936	200	1.0	1.9	0.3	0.2	0.09	3.4	17
	Oct., 1937	248	8.9	2.4	0.3	0.3	0.2	12.1	7
	Oct., 1938	200	15.5	7.7	2.3	0.9	1.2	27.6	15
	Oct., 1939	200	6.7	10.3	3.9	1.7†	3.5	26.1	35
	Oct., 1940	188	0.2	11.9	2.2	4.0†	2.5	20.8	41
Canaan River, Queens Co.	Oct., 1936	196	12.3	2.9	2.1	1.3	0.45	19.1	20
	Oct., 1937	200	20.8	7.8	3.6	2.7	0.9	35.8	19
	Oct., 1938	200	21.8	9.8	10.4	2.9	0.9	45.8	31
	Oct., 1939	200	10.4	16.3	18.6	3.2	2.6	51.1	48
Young's Brook, York Co.	May, 1937	200	8.0	2.4	0.9	0.6	0.6	12.5	17
	May, 1938	200	16.7	4.5	3.0	1.7	0.7	26.6	20
	May, 1939	200	24.0	12.6	15.8	2.7	7.9*	63.0	42
	May, 1940	200	6.5	16.4	19.8	4.3**	6.5	53.5	57
Acadia Expt. Sta., Sunbury Co.	May, 1938	200	2.3	1.8	1.6	1.6	0.06	7.3	44
	May, 1939	200	5.2	2.6	2.3	2.7	0.3	13.1	40
	May, 1940	200	1.4	4.5	3.2	5.0†	0.4	14.5	59

* Many conynmps were diseased at time of spinning, dying later.

** Includes 0.5 cocoons per square foot killed by parasites.

† Includes 0.3 cocoons per square foot killed by parasites.

Population data for a number of plots appear in Table VI. Mortality within the cocoon was quite variable between plots and years, but on the whole, predatory mammals were less important than in Gaspé; predatory insects (elaterids) were about equally important as in Gaspé, except in the McNamee and Acadia Experiment Station plots, where their relative effectiveness was unusually high; other causes of mortality in the cocoon, barring parasites and disease in the later years, were of only minor importance.

TABLE VII
SURVIVAL RATES, SAMPLE PLOTS IN NEW BRUNSWICK

Locality	Year of sampling							Average*
	1934	1935	1936	1937	1938	1939	1940	
Hanwell Road	57	50	—	—	—	—	—	52
Fredericton	67	67	—	—	—	—	—	67
McNamee	25	40	31	37	29	—	—	32
Estey Bridge	—	87	79	—	—	—	—	82
Royal Road	—	79	79	75	64	53	58	60
Canaan River	—	—	43	52	41	40	—	43
Young's Brook	—	—	—	53	45	32**	35 (Spring) 31 (Fall)†	35
Acadia Expt. Sta.	—	—	—	—	36	33	34	34

* Based on totals from all years' sampling.

** Reduction due to death after spinning of 1938 generation material affected by disease.

† Samples totalling 244 square feet taken in general area but not on the standard plot.

Estimates of the percentage survival (emerged cocoons in relation to all empty cocoons) for the different plots are shown in Table VII. The survival rate varied widely between different localities, but variations in different years in a given locality were of a minor degree except in the latest years; disease and cocoon parasites, new elements in the control complex, caused a considerable reduction in survival in the cocoon stage in the Royal Road and Young's Brook plots. On the whole, survival in New Brunswick was at least equal to that in the most favourable locality in central Gaspé, and in most of the New Brunswick plots there was a marked superiority.

Similar estimates for localities in central to northern Maine, based on 1939 population data obtained by American entomologists, appear in the synopsis.

Locality	Source of data	Survival rate		
		Spring samples	Fall samples	Average
Township 12, Range 16	Brown <i>et al.</i> (2)	25	20	23
Township 30	Brown <i>et al.</i> (2)	60	56	58
Aroostook County	Peirson and Nash (4)	61	53	58

With the exception of the first locality, the estimates fall within the range of variations noted for New Brunswick localities.

The infestations in New Brunswick, and in the northeastern states as well, were often quite localized but built up rapidly. Severe defoliation injury frequently resulted within a year or two of the first signs of feeding. The reduction in population level in 1939 and 1940 was due to a combination of circumstances, all unfavourable to the insect. Only small reserves of dormant eonymphs were maintained during the summer season, and there was also a considerable emergence from earlier cocoons of the first generation. Each year a destructive virus disease appeared among the feeding larvae in July, killing a very high proportion of the then existing larval population and practically all larvae ensuing from subsequently emerging adults. Virtually all emergence occurring after June was therefore non-productive. Meanwhile introduced parasites, having built up to effective populations in many localities in south central New Brunswick, were added to the complex of factors affecting the dormant sawflies in the ground.

Diapause and Epidemiology Farther South

The following observations relating to southern New Hampshire and Vermont, tentatively considered in the absence of field records as an area in which a partial third generation probably occurs, are based almost entirely on information supplied by officers of the United States Bureau of Entomology and Plant Quarantine, New Haven, Conn. (2).

Less than 1% of the overwintered cocoons at Wilmington, Vt., persisted in diapause during the 1938 season. This was also the condition in 1939, less than 1% of the overwintered population being unpupated by June 7 (1). Estimates of the degree of diapause at Marlboro, Vt., in 1939, ranged from less than 1% (about 2600 cocoons in soil cages) to about 15% (comparison of spring and autumn population data). At Dublin, N.H., diapause was estimated at 10 to 15%.

Diapause in cocoons of the first generation at Marlboro in 1939 was 12 to 13%.

From these observations it may safely be concluded that large proportions of the overwintered and first generation cocoons produce adults for participation in the seasonal development. During the early part of the season, at least, there is evidently a very small reserve population in diapause.

Survival of cocoons to the time of adult emergence, as based on extensive field counts in the spring and autumn of 1939, averaged 57% at Dublin and 70% at Marlboro.

The infestations in this general area have been more or less localized, but in a number of cases developed with great rapidity to population densities far in excess of those experienced in Gaspé or New Brunswick. Extensive counts of the cocoon population in a woodland near Dublin in 1939 gave a total density of about 160 per square foot; of these, about 85 were living cocoons

in May. As many as 90,000 larvae were counted climbing a single tree during part of the first generation period. In midsummer and later disease swept through the larval population and left practically no survivors. The living cocoons in the autumn were reduced to 11 per square foot.

Extensive samples were also taken at Marlboro in 1939. Here the cocoon density was even greater, about 180 per square foot, but the infestation had apparently been greatly reduced in 1938, since only two cocoons per square foot were living in May, 1939. This was further reduced to 0.4 in October.

Severe defoliation in scattered woodlands throughout the area resulted from the huge populations, but apparently there has been little or no mortality of spruce to date.

The rapid population increases in this area apparently are due to the combination of (a) small degree of diapause in overwintered and first generation cocoons, (b) fairly high survival to the time of adult emergence, and (c) increased number of seasonal generations. At the same time the population is very unstable because of small reserves of cocoons in diapause, and susceptible to violent reduction by factors, such as disease, affecting the feeding larvae.

Discussion

The question of intraspecific differences is of particular interest in this study, and observations bearing on the probable origin of diversity within the species, its perpetuation, and the interaction of climate and diapause as affecting the character of populations in different regions require some further comment.

The origin and subsequent genetic history of the obligatorily parthenogenetic spruce sawfly have been discussed by Smith (5). From a cytological survey of related species of the genus *Diprion*, Smith concludes that the basic chromosome number is seven, and therefore, that both the facultative (6-chromosome) and obligatory (7-chromosome) species formerly included in *polytomum*, arose from a common 7-chromosome prototype, which was a facultatively parthenogenetic (bisexual) species. It is plausible to assume that the obligatory form was more or less heterozygous at origin. Starting out with a certain degree of heterozygosity and possibly accentuated by subsequent mutation, it is inevitable that the species become split into races, due to the self-fertilizing mechanism, inhibition of interbreeding between females and the rare males (infertile triploids would probably result), and the barrier between crossing of the facultative and obligatory forms provided by the chromosome differences. The process of developing a number of homozygous lines would be gradual, but progressive with each generation. Smith found differences in chromosome length, pigmentation of the fifth instar head capsule and gross cocoon weight of European obligatory material, which suggested the existence of distinct lines or races.

In our studies with Canadian obligatory material, we have found differences in the tendency towards diapause and in the proportion of males in pure lines,

which appear explicable only on the basis of racial physiological differences. Higher proportions of males occurred in emergent lines than in diapause lines or in field populations. Smith (5) suggests that variations in male production in different lines is probably due to inherent differences in the factors controlling the speed of budding off of the second polar body from the female pronucleus at the time the latter begins to sink into the yolk; a rapid budding off would result in a male through the failure of auto-fertilization. The higher male production in the laboratory than in the field may be explained as due to the accelerative effect of the higher temperature upon the process of budding off, within limits imposed by the inherent factors controlling the process.

The varied behaviour, with respect to diapause, of different lines reared in the incubator suggests corresponding genetic differences in the factors controlling development, but the *modus operandi* of such factors, in contrast to those of sex determination, is entirely unknown.

The occurrence of emergent and diapause lines in both one-generation and two-generation areas, though in different proportions, indicates that climate has had only a partially selective action in fashioning the composition of the population from the various elements within the species. However, it seems significant that no strongly emergent lines have been found in Gaspé material: at most, only lines that were partially emergent for a very few generations, then terminated altogether due to diapause. From the rearings of emergent lines transferred to Gaspé it is clear that there would be a partial survival of such stock in a one-generation area by virtue of diapause induced by environmental causes.

At the other extreme, diapause lines would appear to have no intrinsic handicap in an area favourable to the development of two or more annual generations, but in view of their slower rate of increase they would in time be greatly outnumbered by emergent strains that made full use of the environmental possibilities.

The environmental causes that induce diapause in emergent stock with advance of the season are obscure, but the adaptive value of the phenomenon is apparent in view of the fact that development is arrested in cocoons of emergent stock spun in late August, under temperature and moisture conditions in the soil about as favourable as those of May and early June, when development is resumed.

Summary

1. The European spruce sawfly is favoured in America by the parthenogenetic mode of reproduction and by the freedom of attack from native parasites. It is well adapted, by means of variations in the diapause phenomenon, to climatic conditions over a wide area and is probably capable of adapting itself to all areas in which spruce grows.

2. The life history and developmental stages within the cocoon are described. The newly spun larva (eonymph) is most commonly affected by diapause and may remain dormant for long periods, up to seven years or more. Eonymphs

in diapause are resistant to factors (except, of course, predatism or parasitism) that cause mortality in later developmental stages.

The advanced larva preceding the pupa (pronymph) may occasionally go into a true diapause, though usually of only a few months' duration.

3. Studies are described which indicate that there are different "lines" within the species, with respect to the inherent tendency towards diapause or continued development; and that the population in southern areas (two or more annual generations) is composed predominantly of "emergent" lines, while the population in northern areas (one annual generation) is composed predominantly of "non-emergent" or "diapause" lines, diapause intervening after a single generation even at favourable environmental conditions.

4. Diapause may be induced in the members of an emergent line by sub-optimal conditions, such as low fluctuating temperature and unfavourable foliage. However, there is no intimate relation between the percentage of diapause in the larvae of an emergent population, and any one of the three factors, temperature, moisture, or food. The only conclusion that can be drawn is that emergent populations are evidently highly sensitive to environmental changes associated with advance of the season. The result is that diapause in the larvae maturing at successive intervals becomes complete at a time when the season is so far spent that a further generation could not develop to maturity. This is the manner by which the insect adapts itself to areas in which more than one generation is possible.

The character of the population in different areas seems to have been fashioned out of the various elements within the species, by the interaction of climatic and genetic factors with respect to diapause and survival.

5. On the basis of biologic and climatic data the eastern part of Canada and adjoining territory in the United States have been divided into zones, representing one-, two-, and three-generation areas; intermediate transitional zones are also indicated. Though much of the zonation is admittedly tentative, it will serve to focus attention upon various aspects of the bioclimatology that require further study.

6. Laboratory and field studies of the factors influencing the resumption of development are described. With Gaspé (one-generation) material, there was typically a progressive response to favourable developmental conditions as the period of cold rest was extended to three or four months. New Brunswick (two-generation) material gave a high response after two months of cold rest. The degree of cold was unimportant, provided it was below the threshold of development.

An incubation temperature of 74° to 75° F. promoted a higher development than one of 65°, but there was little difference in the percentage of development over the limits of 65° to 45°, the latter being close to the threshold of development.

The percentage of development after appropriate cold rest and at an incubator temperature of 74° to 75° was in all cases much lower in unsaturated

atmosphere than at 100% relative humidity. Contact water was not essential to secure very high development in New Brunswick material incubated at this temperature, but in most cases it was necessary to secure high development in Gaspé material. Effective methods of providing contact water include incubation on moist sand or filter paper, frequent momentary dippings, or immersion for one to three days.

The cocoon has an important bearing on water exchanges. It appears to be the only mechanism possessed by the larva to conserve its water supply, since a fatal loss of water occurs when the larvae are removed from the cocoon. (It may have other important functions as well, e.g., respiration, since naked larvae at 100% relative humidity die before water loss reaches fatal proportions.) The protection against water loss afforded by the cocoon is only relative, however, since the rate of loss is nearly proportional to time and saturation deficiency.

The larva absorbs water taken up by the cocoon wall at contact. Absorption is slow since the inner lining of the cocoon is relatively impervious. Preliminary drying may cause a greater water loss than can be compensated for by a brief contact with water, and drying after contact removes the water held in the cocoon wall.

In controlled experiments there was a progressive decrease in the percentage of development in relation to the degree of drying either before or after contact of the cocoons with water.

Temperature fluctuations within the limits experienced in the natural environment appear to have little or no direct influence on the percentage of development in overwintered cocoons. Moisture deficiency in May and June may greatly retard development in the overwintered cocoons in south central New Brunswick, but the insects in diapause respond to rainfall later in the season. Although development in the overwintered cocoons in Gaspé may be inhibited by deficient moisture in the spring, the population does not respond to any appreciable extent to plentiful moisture later in the season (July onwards); it is therefore possible to estimate percentage of seasonal development several weeks before emergence begins.

The characteristically small percentage of development in populations of one-generation areas can only be attributed to the inherent tendency towards diapause, the temperature conditions in the natural habitat failing as a marked stimulus for resumed development.

7. The character of sawfly infestations depends upon several factors which vary in degree throughout the distribution range in North America, viz.: (1) degree of diapause in overwintered cocoons; (2) duration of diapause; (3) number of seasonal generations; and (4) survival of cocoons to the time of adult emergence. Data showing the variability of these factors in a one-generation, a two-generation, and an area probably having a partial third generation, are included, and the nature of the infestations is described. Infestations in northern localities develop slowly but persistently, those in southern localities have violent oscillations in population level.

8. Reproductive capacity of the spruce sawfly is correlated with size, and both are influenced by feeding conditions during larval development, being greater on white spruce than on black spruce. Under very satisfactory conditions, reproductive capacity is approximately 60, but reductions of 30 to 40% may result from food shortage.

The influence of prolonged diapause (four to five years) upon reproductive capacity is small and masked by errors of random sampling. The apparent lack of influence is explicable on the basis of very low metabolic demands upon the stored nutriment during the period of diapause.

Acknowledgments

The writer is indebted to Mr. J. J. de Gryse, Chief of Forest Insect Investigations, Department of Agriculture, Ottawa, and to Mr. R. E. Balch, Entomologist-in-Charge, Dominion Entomological Laboratory, Fredericton, N.B., for advice and encouragement during the course of the studies described in these papers. Many persons associated with the spruce sawfly investigations in Canada have contributed to different phases of the investigations. The field studies in New Brunswick and at Parke Reserve, Que., were carried out under the direction of Mr. R. E. Balch, by the following investigators: Dr. C. E. Atwood (1933 to 1936), C. C. Smith (1936 to 1940), W. A. Reeks (1934 to 1940), R. F. Morris (1938 to 1940), and A. R. Gobeil (1935 and 1936). The pure line rearings in the laboratory were carried out principally by C. C. Smith. In the Gaspé field studies, and in the investigations relating to the breaking of diapause, development within the cocoon, water exchanges, reproductive capacity in relation to diapause, and related phases, the writer was assisted by various workers, particularly J. S. Prebble (1938) and G. W. Barter (1939 and 1940).

The bulk of the data used in the proposed zonation was provided by Dr. A. W. A. Brown, in charge of the Forest Insect Survey for Canada and co-author of the section; in addition, thanks are due to Mr. L. S. Hawboldt, in charge of the Forest Insect Survey in the Maritime Provinces, and to Messrs. A. R. Gobeil and R. Lambert of the Service d'Entomologie, of the Province of Quebec, for records from New Brunswick and Quebec.

Particular thanks are due Messrs. R. C. Brown, P. B. Dowden, and W. F. Sellers, of the United States Bureau of Entomology and Plant Quarantine, New Haven, Conn., for generous permission to use unpublished data obtained by these investigators in Maine, New Hampshire, and Vermont.

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